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The origin of the metabolic fecal nitrogen in relation to protein requirements

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INTRODUCTION

In the last few years, many new protein feeding standards (Burroughs et al., 1974; Satter and Roffler, 1975; Roy et al., 1977; Kaufmann, 1977; Fox et al., 1977) have been developed for cattle and sheep. Basically the difference between the currently accepted systems, such as that published by NRC (1976), is that the new systems recognize the importance of amino acids and not just crude protein (CP) calculated as nitrogen times 6.25. Each system, in its own manner, fractionates the dietary nitrogen. In general, the fractions include undegraded dietary protein, ruminal microbial protein, ammonia nitrogen, absorbed nitrogen, tissue or product protein, waste nitrogen, and maintenance nitrogen. Many variables affect each of these fractions, and estimates have been used in the respective systems to quantitate the nitrogen in each fraction. In order that one system, or a combination of systems, can be adapted for general use in ruminant feeding situations, more critical quantitative estimates are needed for all fractions. The present work had as a general objective to define more precisely a portion of the maintenance nitrogen fraction.

In the original development of the metabolizable protein-urea fermentation potential system as Iowa State University, a value for the metabolic fecal nitrogen (MFN) was needed. The MFN has classically been defined as that nitrogen voided in the feces by an animal on a nitrogen free diet. Literature searches revealed numerous values for ruminants

and monogastric animals. However, a constancy was discovered in that the ruminant values were two to three times greater than those for monogastric animals when expressed on a dry matter (DM) intake basis. Most nutritionists have felt that the MFN voided originated from spent intestinal enzyme nitrogen, cellular debris nitrogen, and intracellular nitrogen. Therefore, based on the above concepts, there should be no difference between the ruminant and monogastric MFN values. The value used in the metabolizable protein system was the monogastric value of 2 mg per g DM intake.

However, these differences stimulated consideration as to their physiological basis. Knowledge of normal ruminant vs normal monogastric animal diets indicated a higher fiber intake with ruminants. The presence of a microbial population in the lower bowel coupled with the higher fiber intakes began to provide some insight as to the basis for the differences observed. Drawing upon accepted concepts in ruminant nutrition, not known when the MFN concept was developed, it was realized that any nitrogen voided in the feces when feeding a nitrogen free diet would be influenced by:

1) the microbial population of the lower bowel,
2) amount of fiber presented to the microbes, and thus, affecting fermentation rates,
3) the preferential need for nitrogen by the microbes at the expense of the host animal, and
4) the recycling of urea into the lower bowel as occurs in the rumen.

With these factors in mind, the present research was initiated to investigate the origin of the MFN in relation to the protein needs of the animal.

LITERATURE CITED

Usage of Metabolic Fecal Nitrogen

According to Mitchell (1964), present day protein biological evaluations can be traced back to Carl Voit's determination in 1872 that gelatin would not support tissue growth. Mitchell also stated the first quantitative studies with vegetable proteins were conducted by Osborne and Mendel in the years between 1911-1920. Their method of evaluation eventually became known as the protein efficiency ratio and was a ratio of body weight gain to protein consumed. Probably the first use of what is known today as a nitrogen balance trial was by McCollum (1914). This technique allowed for a determination of nitrogen losses in both digestion and metabolism. In contrast to the protein efficiency ratio, this method was more time consuming, but produced more detailed results. In order to account for exact values for the nitrogen losses, determination of fecal and urine nitrogen was required at both the "normal" feeding level and at zero nitrogen intake.

Composition of the feces excreted was considered as early as the 1860's when Parkes (1867) examined the excrement of men fed low nitrogen diets. MacNeal *et al.* (1909) and Osborne and Mendel (1914) observed a high proportion of bacterial cells in human and rat feces. Also found were residues of nitrogenous substances that originated from the animal's body proper. Materials such as bile residues, digestive enzymes, cellular contents, and cellular wall debris have been mentioned by Mitchell (1926).

Mendel and Fine (1912) coined a term to describe the body losses of these products when no protein was fed. The principal term used, and the concept most investigated over the years, has been the metabolic fecal nitrogen (MFN), although metabolic fecal fat and metabolic fecal minerals have been described also. As explained in detail by Mitchell (1926), this term is really a misnomer. The fecal nitrogen excretion is not as closely related to body weight or surface area as is energy utilization. Fecal nitrogen losses from feeding a nitrogen free diet are mainly the result of the dry matter consumption and indigestible constituents of the diet and are approximately 2 mg of nitrogen per g dry matter (DM) consumed. Therefore, these nitrogen losses are more a product of digestion than metabolism, and as such, are charged as an expense to the food nitrogen consumed. However, nutritionists, then as well as now, talk of this loss as a maintenance loss of nitrogen.

The other route of nitrogen loss when no nitrogen is fed is via the urine. A classical piece of research that set the stage for present day protein nutrition was reported by Folin (1905) who described the nitrogen constituents of this loss. In a broad sense, Folin characterized the urinary nitrogen into endogenous and exogenous fractions. The endogenous fraction represented the nitrogen excretion due to the constant turnover of body protein. This fraction contributed a constant amount of nitrogen to the urine each day, regardless of diet. The origin of the exogenous fraction was dietary protein exclusively.

Therefore, the amount varied depending on nitrogen intake. Later, Schoenheimer et al. (1939) reported Folin's exogenous nitrogen did actually originate from body protein. Still later, Mitchell (1955), using data from his coworkers (Burroughs et al., 1940), proved Folin's endogenous nitrogen was constant and not altered by the feeding of single amino acids or mixtures of amino acids over a range of intakes. This conclusion did not disagree with Schoenheimer's dynamic movement of nitrogen between dietary nitrogen and body nitrogen, with the excess excreted in the urine. However, in a final or end-product sense, it agreed with Folin's concept that endogenous nitrogen originated from a dietary source, and that the endogenous and exogenous nitrogen metabolism were independent.

Thomas in 1909 (Mitchell, 1964), unaware of the yet to be proposed concepts above, began the development of a protein evaluation method that nevertheless had its roots buried deeply in those concepts. Proposed by Thomas was a biological value determination for protein in which the relative amount of absorbed protein to protein retained would be determined. This proposal was a bit "deeper" than previous ones in that the protein was given a value not only for growth, but also for maintaining the body's nitrogen integrity. After making this proposal, Thomas seemed to lose his interest in protein evaluations as little can be found in the literature concerning subsequent protein research by Thomas.

Fortunately, about 14 years later, Mitchell (1924) described a refinement of Thomas' method that resulted in direct fecal and urine

total nitrogen determination and indirect determination of dietary nitrogen in either excrement. Essentially, the concept provided an estimate of the percentage of absorbed nitrogen not appearing in the urine. Calculation of the biological value (BV) of a protein became:

$$BV(\%) = \frac{\text{N intake} - (\text{fecal N} - \text{metabolic fecal N}) - (\text{urinary N} - \text{endogenous urinary N})}{\text{N intake} - (\text{fecal N} - \text{metabolic fecal N})} \times 100$$

where: 1) N is symbol for nitrogen,

2) N intake, fecal N, and urinary N are measured during the period of feeding the protein being tested, and

3) metabolic fecal N and endogenous urinary N are measured during a period of zero N intake.

This equation has now come to be known as the Thomas-Mitchell method for biological value determination. The equation adds back to the nitrogen intake of the test protein those losses of nitrogen associated with maintenance excluding losses in hair, sweat, skin, etc. Therefore, the test protein was credited not only for its growth promoting abilities, but also for the ability to satisfy maintenance and digestive nitrogen losses. This meant that the MFN and endogenous urinary nitrogen (EUN) must be accurately measured for detailed biological value determinations. In order to overcome intake problems with diets containing no nitrogen, Mitchell and Carman (1926) began to determine MFN and EUN when feeding diets containing a low

level of egg protein, approximately 4% crude protein (N X 6.25). The assumption was that all the egg protein was digested, and hence, made no contribution to the fecal nitrogen value. Also assumed was that the absorbed egg amino acids were perfectly utilized, and the urine nitrogen represented a true EUN value. Therefore, these results were considered to be the same as what would have been determined had a zero nitrogen diet been fed.

Therefore, MFN losses were originally, and still are, used in the determination of biological value. Many proteins have been evaluated mostly by Mitchell and his graduate students while at the University of Illinois and after leaving Mitchell's laboratory. These values have been routinely used by nutritionists for development of adequate protein levels in diets for both man and animals. Consequently, to this time, the MFN values have been considered only in light of their need for the biological value determination.

Metabolic Fecal Nitrogen Determinations

The classical method of MFN determination was to measure the fecal nitrogen excretion when a zero protein diet or one containing a small amount of a highly digestible protein was fed. There were problems with this approach mainly as regarding feed intake. Therefore, researchers began to use a method of extrapolating back to zero nitrogen intake when plotting fecal nitrogen excretion against nitrogen intake (Titus, 1927). The intercept of this line with the fecal nitrogen

axis then represented the MFN value. The modified procedure simplified matters as the experimental animals could be fed their test diet at an adequate nitrogen level and still serve to estimate the MFN.

Determination in monogastric animals

In order not to confound effects, initially MFN values will be referred to when determined with diets containing no protein or a small amount of highly digestible protein and little indigestible carbohydrate. Consideration will initially be given to direct determinations.

Mitchell (1926) reported values for MFN determined by many different laboratories. Across 22 experiments with humans, eight researchers determined the mean value for MFN to be 2.32 mg per g DM consumed. Across 10 different trials mentioned in the same article, a value of 1.96 mg MFN per g DM consumed in dogs was reported.

Mitchell (1924), in his paper describing his modification of Thomas' biological value, reported an average of 1.88 mg MFN per g DM consumed with 212 rats in his laboratory. Mitchell and Carman (1924) reported values of 1.60, 1.80, 1.67, and 1.65 mg MFN per g DM intake when feeding nitrogen free diets. Bosshardt and Barnes (1945) using mice found values for MFN of 3.00 mg per g diet when the mice were fed at "30% of their normal caloric intake." As will be noticed as this discussion continues, the value of 3.00 mg MFN per g diet intake is about 50% higher than other values. The restricted intake is one possible reason. Several experiments by English workers with rats

(Bartlett et al., 1938; Macrae et al., 1943; Henry and Kon, 1946) resulted in an average MFN value of 2.23 mg per g DM consumed. Mitchell and Bert (1954) reported results of several experiments conducted at the University of Illinois. A value of 1.44 mg MFN per g DM consumed was found when feeding a low nitrogen diet. A similar value in rats of 1.38 mg MFN per g DM intake was reported by Meyer (1956).

More recently, Harmon et al. (1968) determined MFN with rats fed egg protein to be 1.20 and 1.74 mg per g DM intake in two experiments. A liquid casein hydrolysate plus amino acid diet was also fed in two trials and resulted in MFN values of 3.38 and 2.51 mg per g DM consumed. These higher values are probably a reflection of the diet. Several research reports mentioned by Eggum (1973) presented MFN values in rats ranging from 1.82 to 2.90 mg MFN per g DM consumed with an average of 2.25 g. However, no information was given for any experiment as to protein level or amount of indigestible carbohydrate fed. Eggum's research resulted in a MFN value of 2.04 mg per g DM intake.

A simple calculation of the mean of the above values without regards to number of experiments comprising each value results in an average MFN value of 2.06 mg per g DM consumed in rats. Later, this value will be compared to that determined in other animals.

Similar experiments with swine have been conducted to directly determine MFN. McCollum and Steenbock (1912), using data from seven experiments, reported an average MFN value of 1.50 mg per g DM consumed. Mitchell and Bert (1954) cited data by Schifton, 1932, in

which case MFN was determined to be approximately 1.0 mg per g intake of dry diet. The next year, a value of 1.14 mg per g DM consumed was reported as being determined in Mitchell's laboratory (Armstrong and Mitchell, 1955).

Canadian workers (Whiting and Bezeau, 1957a) presented MFN results which averaged 0.86 mg per g DM intake. Eggum (1973), again with no mention of level of protein or amount of indigestible carbohydrate fed, reported the MFN range in 15 different experiments was 0.90 to 1.99 mg MFN per g DM consumed. A MFN value range of 0.64 to 1.21 g per g DM consumed was determined in Eggum's own research in three different trials using the same pigs in each trial. The wide range was probably due to an increased feed intake as the pigs became heavier. Across those trials, the values averaged 0.84 g MFN per g intake of DM.

In contrast to the report by Mitchell (1924), it appears that swine, when fed zero or low protein diets with no indigestible carbohydrates, excrete less than 2 mg MFN per g DM intake. A simple averaging of the values cited in the reports above, without regard to number of experiments, results in a MFN value of 1.18 mg per g DM intake. Interesting is the fact that R. M. Forbes, 1956, quoted this exact figure to Lassiter *et al.* (1956), and it was then used in their calculation of biological values. At this point, it is unclear as to the reason for this difference from the somewhat established 2 mg per g diet DM (Mitchell, 1926) and the 2.06 mg MFN per g DM intake computed from reports previously reviewed with rats.

One possible explanation lies in the second determination method mentioned earlier; extrapolation to zero nitrogen intake. This procedure was first proposed by Titus (1927) as more exact than the direct determination. Those results will be reviewed later, but basically, the proposal was that the extrapolation method would provide a MFN value more representative of the actual dietary influence on fecal nitrogen excretion. Therefore, any influence due to indigestible carbohydrate would be present; its exact role in MFN will also be considered later.

Consideration will now be given to the MFN value for rats determined by extrapolation on diets low in indigestible carbohydrates. Not a great deal of information is available for the comparison of the direct method vs extrapolation method in the same experiment. However, Bosshardt and Barnes (1945) used mice fed diets ranging from 0 to 40% egg protein. By not including the zero egg protein diet, a linear function resulted when plotting mg fecal nitrogen per 100 g food intake vs g nitrogen per 100 g food. Extrapolation to zero nitrogen intake resulted in a value of 3.23 mg MFN per g food consumed. Previously, it was discussed the zero protein diet in this experiment resulted in a value of 3.00 mg MFN per g food intake. The difference between the two values was statistically significant. Mitchell and Bert (1954) conducted a similar experiment with a large number of albino rats. The extrapolated MFN value was determined to be 1.33 mg per g DM intake when a 0.042% nitrogen diet was fed. The 0.042% diet actually

resulted in a value of 1.44 mg per g DM. The researchers stated that statistically there was no difference between the two values. Harmon et al. (1968), upon regressing the ratio of fecal nitrogen/dry matter intake on the protein content of the diet, developed a series of equations from which the MFN could be determined. Regardless of the experiment and type of diet, the calculated MFN values were almost exactly the same as those determined directly and reported earlier in this discussion. Therefore, with this limited amount of data, it would appear that MFN determined in rats by extrapolation is a satisfactory method. If the MFN does consist of a constant fraction not dependent on intake, as suggested by Schneider (1934, 1935), and is therefore a part of the endogenous nitrogen, one would not expect a change in endogenous nitrogen excretion, including MFN, when protein was fed (Burroughs et al., 1940). Although, as detailed in Schneider's papers, a more likely explanation is that the rats were fed within a normal range of intake. Therefore, the constant fraction becomes quite small, and the MFN is then dependent on DM intake. The exception to the extrapolation method for satisfactory MFN determination was the work by Bosshardt and Barnes (1945). However, in another portion of that paper, there seemed to be an indication by those researchers that the difference was due to the restricted intake of the mice.

In swine, a comparison of direct vs extrapolated determination becomes confounded with indigestible carbohydrates fed with the increasing protein levels. The indigestible carbohydrate effect will be

discussed more specifically in a later section. Using pigs of approximately 34 kg live weight, Bell et al. (1950) determined by extrapolation that the MFN value was 1.8 mg per g DM consumed. These diets contained at least 5% crude fiber. Whiting and Bezeau (1957a) fed 5% Solka floc in a semipurified diet containing essentially no protein. The MFN value determined with this diet was 0.87 mg MFN per g DM intake. This was not statistically different from that determined by extrapolation when increasing levels of nitrogen were fed, 1.02 mg MFN per g dry matter intake. Using diets that contained 6% crude fiber, Armstrong and Mitchell (1955) extrapolated to nearly zero nitrogen intake and determined 1.01 mg per g of intake on a DM basis to be the MFN value. The determined value at the same level of nitrogen intake was 1.14 mg MFN per g DM intake. Mitchell (1964) reported several equations for fecal nitrogen depending on the dietary protein content of the diet. At zero nitrogen intake with swine, the MFN became 2.65 mg per g DM consumed. These data were taken from a monograph by Schneider, 1947, which contained a discussion of "normal" feeds. Therefore, it is safe to assume some feeds on which the equations were based had higher levels of crude fiber.

In all reports where MFN was determined by both methods, and in a comparison of the average of the extrapolated values, it can be demonstrated that the extrapolated value is usually higher. As indicated earlier, this higher value may be due to the use of indigestible carbohydrates in the swine diets in contrast to none in the rat diets.

Therefore, the MFN tends to be more constant in rat experiments, while in swine experiments varies due to a constant fraction plus a fraction dependent on varying dietary factors.

Determination in ruminants

Studies with ruminants to determine their MFN are somewhat biased by the facts just discussed with swine. Due to the experimental unit, whether it be cattle, sheep, or goats, some indigestible carbohydrate is usually always fed, except with the nursing young. Therefore, this can influence the MFN determination. As was done in the Literature Review section for monogastric animals, direct determinations of MFN will be given first consideration.

Using diets containing 15% crude fiber, Sotola (1930) determined the MFN of cattle to be 6.53 mg per g DM intake. Turk et al. (1934) conducted two trials with lambs fed 40% wheat straw and 5% cellulose. The MFN values averaged 5.08 and 5.12 mg per g DM intake in the two trials. The diets used contained 1.63 to 1.92% CP. In a similar experiment reported the next year (Turk et al., 1935), the fiber content was reduced to 25% wheat straw and 10.5% cellulose, and the diet had a CP content of 1.15%. A similar, but higher, MFN value of 6.29 mg per g DM intake was determined in this second experiment. No explanation, except experimental variation, was provided for the difference. Hutchinson and Morris (1936) determined the MFN loss in both goats and cattle. When feeding an almost nitrogen free diet containing 40 to 47% oat straw or sawdust to goats, the feces contained 5.00 mg nitrogen per

g dietary DM. Adding cellulose to the diet in a separate experiment increased the MFN value from 4.68 to 5.28 mg per g dietary DM. With a cow eating 6.8 kg per day of a low nitrogen diet, 4.50 mg MFN per g DM intake was excreted. The authors made a statement that the MFN of ruminants was about twice that of monogastric animals and was probably due to the intake of a larger quantity of indigestible carbohydrate in ruminants. Although the effect of indigestible carbohydrate on MFN was known, this was one of the first statements about the magnitude of its effect in ruminants. By feeding sheep a low nitrogen (0.14%) diet containing 25% wheat straw and 10.5% wood pulp, Harris and Mitchell (1941) determined a MFN value of 5.55 mg per g DM consumed, which is similar to the others reported above.

Mukherjee and Kehar (1949) conducted an extensive study of the MFN excretion of cattle. In order to reduce variation due to intake differences, any refusals of the diets, especially prevalent with the nitrogen free diet, were force fed to the animals. The nitrogen free diet contained 36% hot-alkali treated bagasse which should have provided less indigestible carbohydrate than diets in other research reported so far. With the 36% bagasse diet, MFN values ranged from 2.00 to 4.20 mg per g DM intake. The average was 3.13 mg MFN per g DM intake. It should be recognized that 3.13 mg MFN per g DM is approximately half-way between the ruminant and monogastric animal values reported previously. Kehar and Mukherjee (1949) in similar experiments used an oat straw and a particular local hay sample that were both low in

indigestible matter. Using diets containing 25% indigestible matter from the straw and hay and having a low nitrogen content, the MFN of cattle was determined to be 2.40 mg per g DM intake, a value very similar to that determined in rats.

All the reports to this point have been conducted with "more or less" mature ruminants. Blaxter and Wood (1951) reported on the first of a series of experiments to determine the nutritional requirements of the young calf. In order to establish protein requirements, MFN was determined feeding a liquid, nitrogen free diet containing predominantly glucose, lard, and minerals. The three calves excreted 4.5, 4.2, and 4.1 mg nitrogen per g DM ingested for an average of 4.27 mg MFN per g DM consumed. That average is quite close to most MFN values determined with mature animals. However, the daily DM excretion and daily apparent DM digestibility were 4 times greater and 20% less, respectively, when compared to the same calves fed a similar diet providing 3.62% nitrogen. This was probably due to the diarrhea experienced by the calves on the nitrogen free diet. Lofgreen and Kleiber (1953) used a slightly different direct determination of the MFN in three day old calves. Using ^{32}P-labelled casein and the N:^{32}P ratio in feed and feces, they determined a value of 2.70 mg MFN per g DM intake. Normal levels of energy and protein (casein) were fed with little indigestible carbohydrate, and therefore, it was stated this value should be more accurate than feeding nitrogen free diets at less than optimum intake. A similar experiment was conducted by Roy *et al.* (1964)

in which they assumed the milk protein fed was 100% digested and absorbed. Using that assumption, the MFN value determined and used in their digestibility work, was 1.91 mg per g DM intake. A group of Missouri researchers (Ellis et al., 1956), using sheep fed a ration containing 0.004% nitrogen and 40.3% Solka floc, determined MFN excretion to be 2.39 mg per g DM intake.

To summarize the direct MFN determinations with ruminants, the values appear to fall into two groups. In the group fed indigestible carbohydrates, the MFN value averaged approximately 5.37 mg per g DM intake. With the other group in which no or little indigestible carbohydrate was fed, the MFN approached the value found in rats. The average was approximately 2.80 mg MFN per g DM fed which was almost half the average for the other group and only 0.74 mg per g DM above the value for rats reported previously. These data were considered in the decision to use the value of 2.0 mg per g DM in the Iowa State University metabolizable protein-urea fermentation potential concept of protein nutrition for ruminants (Burroughs et al., 1974; Burroughs et al., 1975).

As was done with the monogastric animal MFN comparisons, consideration will be given to indirect methods of determination in ruminants. Titus (1927) first proposed the extrapolation method. The procedure was to extrapolate back to zero nitrogen intake when plotting fecal nitrogen against nitrogen intake. The data on which the regression equation was based were from diets of adequate energy and nitrogen intake, a "normal" diet. Therefore, the MFN value determined should apply at

the intake and at the level of indigestible carbohydrate in the test diets. Using steers and graded levels of paper pulp replacing alfalfa in the diet, Titus (1927) determined the MFN to be equal to 2.43 mg g DM intake plus 1.56 mg per g fecal moisture. Assuming 70% moisture in the feces, the total MFN becomes 3.52 mg per g DM consumed. Blaxter and Mitchell (1948), in their development of a factorial method for estimating the ruminants' protein requirements, placed a value of 4.50 mg per g DM consumed based on the extrapolation of available data with sheep.

Mitchell (1964) presented two equations expressing the relationship of fecal protein to dietary protein for cattle and sheep. At zero dietary protein the fecal nitrogen values were 4.32 and 4.84 mg per g dietary DM, respectively. An interesting fact also presented is that the MFN value for horses was 4.32 mg per g dietary DM. Even though the horse is a monogastric animal, the normal diet approximates that of a ruminant in regards to indigestible carbohydrates.

Experiments conducted in Utah (Asplund and Harris, 1968) using wethers resulted in a MFN value of 5.64 mg per g DM intake. However, also using sheep, a MFN value of 2.14 mg per g DM intake was determined by Singh and Mahadevan (1969) using the extrapolation method. By direct determination the MFN excretion was estimated to be 2.54 mg per g DM intake of a 0.051% nitrogen diet. By assuming one-third of the DM intake appears in the feces, an estimated 5.88 mg MFN per g DM intake was determined from cattle and sheep data based on 45 digestion trials

(Hironaka et al., 1970). The results from the regression of fecal nitrogen vs dietary nitrogen, both on a per unit of DM intake, did not produce a straight line and were therefore "not suitable for extrapolation."

From data used to investigate urea kinetics in dairy cows, Mugerwa and Conrad (1971) stated that the MFN of these animals was 6.65 mg per g DM intake. This higher value is of interest since the diets used for the basis of the extrapolation contained from 85 to 91% ground shelled corn which is not high in indigestible carbohydrates (ca. 2.2% crude fiber). Strozinski and Chandler (1972) used a modification of the conventional extrapolation procedure in which they fed to bull calves a constant nitrogen and caloric intake with increasing powdered polyethylene. The results were not reported on a DM intake basis, but using their data, an approximate value of 3.27 mg per g DM intake can be calculated. Biddle and Evans (1973), using steers and a depletion technique, regressed ingested nitrogen vs apparently digested nitrogen and extrapolated to a MFN value of 6.20 mg per g DM intake. Stallcup et al. (1975) compiled data from the digestibility trials on 68 forages and computed MFN values based on these data. The values reported were from different methods, and therefore, are hard to interpret in terms of the present discussion. As best can be deduced from these data, the MFN values found were 6.63, 5.84, 6.52, and 5.73 mg per g DM intake.

Across all these MFN determinations in ruminants by the extrapolation method, the average was 4.92 mg MFN per g DM consumed with a range

of 2.14 to 6.65 mg. As indicated at the beginning of this discussion, nitrogen and indigestible carbohydrate content of the rations in the reports presented could not be held as constant as had been done in the discussion with monogastric animals. This undoubtedly was one reason for such a wide range in values.

Swanson (1977) attempted to use only experiments similar in dietary composition in determining MFN from 16 different experiments. By extrapolation, a MFN value of 4.03 mg per g DM intake was determined. The data contained only one of the reports used in computing the above average, but both average values were similar.

In contrast to a similar comparison in the monogastric animal, MFN values by direct determination were higher than the extrapolated values in ruminants. However, a more important concern was the basis behind the widely differing values for the monogastric and ruminant animal. If the MFN loss is one of residues from body secretions and tissue loss incidental to the movement of food down the gastrointestinal tract as Swanson (1977) suggests, then why would there be a species difference when expressed per g DM intake? If one considers the MFN values for young ruminants consuming very little indigestible carbohydrates the MFN value approached 2.5 mg per g of DM consumed, neglecting the one extremely high value reported in the above discussion. Also, the fact that the swine value, when determined by extrapolation, was higher than when directly determined seems to imply the higher level of indigestible carbohydrates had an effect on increasing

the nitrogen loss. Since many nutritionists have accepted the concept that the MFN is a loss of spent enzymes, body secretions, and tissue residues, it has been reasoned that the indigestible carbohydrate had its effect by increasing the scouring of the intestinal wall and/or increasing secretions into the intestines. While these factors may be involved, an alternate explanation is the microbial fermentation in the gut distal to the stomach. The importance of this population will be detailed in subsequent sections.

Effect of Lower Intestinal Microbes on Nitrogen Excretion

The presence of a microbial population in the lower intestine and subsequent voiding of microbial cells in the feces has been known for some time (MacNeal et al., 1909; Osborne and Mendel, 1914). However, not until recently has this population been actively investigated as to its function for or against the host animal. One exception is that it has been known for some time that the large intestine is essential for efficient digestion of roughages by the horse. Much knowledge has been published about the ruminant microbial population and its function, primarily due to its foremost position in the ruminant's gastrointestinal anatomy. This rapid increase in knowledge about ruminal fermentation helped stimulate the investigation of the fermentation in the lower tract. Also, the relationship of the end products of fermentation to certain disorders of the large intestine in humans (Visek, 1972) has surely increased research efforts in this area.

Microbial population of lower intestine

Although microbes are found throughout the gastrointestinal tract, the largest quantity (1 to 3 X 10^{10} bacteria per g wet contents) is in the cecum and large intestine according to Schaedler (1973) and Refat (1978). The lactobacilli and anaerobic streptocci are the first to become established soon after birth in the mouse's stomach and large intestine (Schaedler, 1973). By day 16 of life, the mouse acquires its normal population of anaerobic bacteria. This somewhat coincides with the ingestion of the first solid food.

Bryant (1974) working with humans and swine reported the microflora of the hindgut of both to be somewhat similar to that found in the rumen. Comparison of cecal bacteria numbers reported by Ulyatt et al. (1975) and ruminal bacteria numbers by Hungate (1966) and Church (1975) indicates a similarity in numbers between both fermentation sites; approximately 10^5 per gram of contents. Ulyatt et al. (1975) also indicated that the increase in aerobic and anaerobic bacteria from the duodenum to the rectum of sheep was about 50%. Therefore, the numbers in the small intestine were approximately 10^3 fold less than in the rumen. The anaerobic bacteria made up about 55 to 60% of the total bacteria in the large intestine. The percentage agrees with the counts reported by Bruns et al. (1977). Ulyatt et al. (1975) reported no protozoal population was found in the cecum of sheep and many large bacteria were also absent. The Gram-negative rods predominated with members of the genera Bacteroides, Bifidobacterium, Butyrivibrio,

Fusobacterium, and Lactobacillus as the primary bacteria present (Mann and Ørskov, 1973). Also indicated was a small proportion of bacteria belonging to the genera Streptococcus, Peptostreptococcus, Selenamonas, and Micrococcus. Raibaud and Ducluzeau (1973) reported that a normal rat's digestive tract should contain bacteria from the following genera: Streptococcus, Eubacterium, Zymbacterium, Pasteurella, Ristella, Veillonella, Clostridium, Inflabilis, and Acuformis. Chawla et al. (1976) reported anaerobic gram positive rods and cocci predominated in the control rats fed no antibiotics. Bruns et al. (1977) also reported finding Lactobacillus acidophilus, L. bifidus, Escherichia coli, and Enterobacter aerogenes in the cecum of rats. With the exception of a few genera listed by Raibaud and Ducluzeau (1973), most genera indicated have been found in the rumen (Hungate, 1966; Church, 1975).

Microbial nitrogen fermentation in lower intestine

Ørskov et al. (1970) and Hecker (1971a) both reported that the cecal fermentation appears to be similar to that in the rumen. Therefore, if the cecal and ruminal bacterial population and resultant fermentation are similar, then the question arises as to whether or not one can assume the microbial metabolism of nutrients, more specifically nitrogen, to be similar. In order to shed light upon this relationship, consideration will be given to both ruminants and nonruminants together. However, any microbial products from the rumen will be ignored in order to reduce confusion as to the origin of fecal microbial protein.

There are two general sources of nitrogen available in the rumen to bacteria; nitrogenous material entering via the esophagus and nitrogenous compounds entering from the blood. Naturally, the esophagus provides nitrogen from saliva, whose nitrogen constituents originally came from the blood, and ingested feed. One source of nitrogen for the lower intestinal bacteria is undigested dietary nitrogen, enzyme nitrogen, intestinal secretion nitrogen, and intestinal cellular debris nitrogen from the chyme (Mitchell, 1926) as it moves down the tract.

Work by Nasset and Ju (1961) and Twombly and Meyer (1961) point out that the exogenous nitrogen is diluted to a considerable extent by the endogenous nitrogen secretions. Nasset and Ju (1961) indicated that as much as a sixfold dilution occurred when determined with dogs and rats. However, their value may be inflated due to the experimental procedures, and a more realistic figure was determined by Nasset (1957) to be about a one to one dilution. However, Crompton and Nesheim (1969) later found only a 50% dilution of exogenous protein with endogenous protein in ducks. By feeding a protein free diet to rats, it was found that 10 to 16 mg of nitrogen would be present at any given time in the intestine from 0 to 12 hours postfeeding (Twombly and Meyer, 1961). These values are about half of those found by Geiger _et al._ (1958) using rats also. Upon extrapolation of the data of Zebrowska and Buraczewska (1972b), the endogenous nitrogen flow through the duodenum was 8 mg per hour. By comparing the intestinal nitrogen with the protein free diet to the intestinal nitrogen of diets containing

increasing egg protein, the data of Twombly and Meyer (1961) indicate an approximate one to one dilution with a 10% CP diet one hour after feeding. The 5%, 15%, and 20% CP diets resulted in a 50%, 120%, and 220% increase, respectively, in intestinal nitrogen above the endogenous level. After eight hours postfeeding, when it could be expected that all the egg nitrogen was absorbed, the values were 50%, 63%, 103%, and 106% above the endogenous level with the 5%, 10%, 15%, and 20% CP diets, respectively. Therefore, these data show that not only is there a background level of endogenous nitrogen in the gut, but also that there is an increasing response to dietary protein. Refat et al. (1977) reported a relative constant supply of amino acids in the portal vein of pigs fed protein free diets. The absorbed amino acids amounted to approximately 15 g for seven hours after feeding. Those researchers pointed out the importance of the intestinal endogenous nitrogen being a mechanism to balance the amino acids available from the diet. From the results of Crompton and Nesheim (1969), it appears that dietary proteins are digested more rapidly than the endogenous proteins. This finding was confirmed by Zebrowska and Buraczewska (1972a). Therefore, not only are there large quantities of endogenous protein in the gut, but the endogenous protein makes up a large portion of the nitrogen that reaches the large intestine.

The second source of nitrogen available to rumen microflora is from the blood system. As mentioned earlier, saliva enters the rumen in association with the actual feed, or due to a feeding reflex, but

the nitrogen constituents did have the blood as their source (McDonald, 1948; Somers, 1961). Another route of nitrogen entry into the rumen is a direct transfer across the rumen wall as urea (Houpt, 1959; Packett and Groves, 1965; Houpt and Houpt, 1968). Currently, interest seems to center around the factors limiting this ruminal transfer. Some researchers contend the transfer to a simple diffusion process from the blood to the rumen (Cocimano and Leng, 1967; Houpt and Houpt, 1968; Ford and Milligan, 1970). Others feel the transfer is limited at high levels of blood urea (Weston and Hogan, 1967; Vercoe, 1969). Although it can not be stated with surety, these investigations, along with the developing interest in cecal fermentation, must have led researchers to consider similar transfers in the small and large intestine (Nolan and Leng, 1972; Nolan et al., 1973; Mazanov and Nolan, 1976; Nolan et al., 1976; Okumura et al., 1976). Approximately 76% of the apparently degraded urea in the ruminant digestive tract was degraded by microbial fermentation postruminally according to Nolan and Leng (1972). In further research, these researchers (Nolan et al., 1976) found approximately the same amount of nitrogen from blood urea degraded in the digestive tract (5.3 g nitrogen per day), but had narrowed the postruminal influence down to 25% of the degradation occurring in the cecum. Interestingly, the rumen only accounted for 20% of the urea degradation. The total ammonia produced in the cecum amounted to 4.8 g nitrogen per day of which 4.2 g left the cecum. Of the ammonia lost from the cecum, 50% (2.1 g) was derived from nitrogenous compounds other than blood urea or ruminal microbial nitrogen.

Based on the data of Nolan et al. (1976), 1.6 g of the dietary nitrogen bypassed the rumen, and if 90% of that nitrogen was digested postruminally (Burroughs et al., 1975; Mason and Frederiksen, 1979), then only 0.16 g of dietary nitrogen would be presented to the cecum. Therefore, if it was 100% deaminated, a conservative estimate of the ammonia nitrogen lost from the cecum, derived from bacterial fermentation of intestinal endogenous nitrogen, would be 1.94 g (2.10 - 0.16 = 1.94) per day. Expressed another way, 144.2 mg of ammonia nitrogen per kg metabolic weight (32 kg live weight) were lost from the cecum due to fermentation of the endogenous nitrogen. Based on a calculation from the data of Twombly and Meyer (1961) with a 20% crude protein diet and 175 g rats, the digestive tract produces approximately 120 mg endogenous nitrogen per kg metabolic weight. Although there is ample chance for error in the assumptions and calculations above, the results do point towards a large destruction of endogenous protein by the bacteria in the lower intestine, especially the cecum. The magnitude of this liberation of ammonia was demonstrated by Faichney (1968) in which the cecal ammonia content was approximately three times that in the rumen. Also, there tended to be a constant level of ammonia in the cecum with only a slight increase 12 to 16 hours after feeding. If the ammonia produced is converted to urea and excreted in the urine, or if it is used for microbial growth in the lower intestine, the animal essentially suffers a loss of a needed nutrient.

A current topic of interest to swine nutritionists is the availability of amino acids. Amino acid availability relates to the present discussion as any degradation of dietary or intestinal endogenous nitrogen and utilization for microbial protein synthesis could completely change the fecal amino acid composition (Zebrowska et al., 1978a). Mason (1969) concluded that when ruminants were fed normal diets undigested dietary nitrogen, water soluble nitrogen, and nitrogen of body origin were quantitatively unimportant compared to the fecal bacterial residues. It was strongly suggested most of these bacterial residues originated in the rumen. However, work reported by the same author (Mason et al., 1976) with pigs demonstrates that the fecal nitrogen of monogastric animals could contain large numbers of bacterial residues. The amino acid patterns of the whole feces, isolated bacteria, and as reported by other workers, the MFN (protein) from rats and pigs was quite similar. The mean ratios of the total amino acids in whole feces to isolated bacteria, in MFN to whole feces, and in MFN to isolated bacteria equaled 0.991, 1.048, and 1.032, respectively. Therefore, Mason et al. (1976) suggested that the intestinal bacteria could influence MFN values. Mason et al. (1977) concluded the microflora of the hindgut of sheep have more than adequate levels of nitrogen in relation to energy. Therefore, fermentation in the large intestine could modify the fecal nitrogen composition in sheep, and possibly the MFN loss also. Recently, Mason (1979) proposed factors that would influence fecal nitrogen losses in light of his previous research. These factors will be

discussed in detail later; however, all his papers demonstrate that the lower intestinal microbes can and do modify nitrogen entering the lower intestine. Whether this nitrogen be from dietary sources, intestinal endogenous nitrogen, blood urea, or rumen bacteria in the ruminant, it will probably be modified before appearing in the feces. As indicated above, swine nutritionists are concerned about this fermentation effect on the determination of amino acid availability. However, this may or may not affect the magnitude of the MFN.

The use of germfree animals or animals whose intestinal population has been modified by antibiotics may provide information as to the effect of the lower intestinal fermentation on MFN excretion, and nitrogen loss in general.

Comparison of conventional vs modified animals

Microbial population Conventional animals have a normal intestinal microflora population. Animals can be germfree in which case they are raised in insolators in a sterile environment. Another manner in which to approach this particular situation is to modify the population. Most commonly, this simpler procedure is used to produce a situation somewhat similar to that in a germfree animal in reducing intestinal fermentation effects. The procedure involves feeding high levels of unabsorbable oral antibiotics. In general, the literature indicates this does not completely eliminate the intestinal bacteria. As an example, Kent et al. (1969) indicated an antibiotic supplement of neomycin sulfate, polymyxin B sulfate, and bacitracin would eliminate

E. coli and enterococci from the cecum and eliminate from the small intestine bacteroides, enterococci, and lactobacilli. An increase was found in proteus in the cecum, but no change occurred in the small intestine. However, germfree and antibiotic modified animals have been shown to be somewhat similar in regards to excretion products (Visek, 1978). Therefore, in this section of the Literature Review, the germfree and antibiotic modified animals will be combined as if they are partially interchangeable, but realizing the antibiotic modified animals will have somewhat less response than germfree animals when both are compared to conventional animals.

Growth rate Since the discovery of the growth promoting effects of antimicrobials in the mid-1940's (Francois, 1959), there have been numerous reports on the effect of antibiotics on growth and on the mode of action involved (Jukes and Williams, 1953). Pecora (1953) fed rats a high level of rice starch plus lysine and threonine and one of the following antibiotics: penicillin, aureomycin, streptomycin, terramycin, bacitracin, or chloromycetin. Growth rate was increased about 25 to 30% for 9 weeks with the two most effective antibiotics. Carcass weight was also increased with antibiotics. It is interesting to note that MFN values calculated from the data indicated an 18 to 22% decrease with antibiotic supplementation. Forbes (1954) observed similar results when feeding rats soybean meal with and without streptomycin and chloromycetin. The additional rate of gain with antibiotics was maintained for 7 weeks. Levenson and Tennant (1963) found a greater body and

carcass weight with germfree mice vs conventional mice or E. coli monocontaminated mice in two different experiments. However, the results were not consistently significant within experiments, but the germfree animals never weighed less than either of the other groups. Growth rate and final body weight were similar between germfree rats and rats "conventionalized" after weaning. Carcass weight was less for germfree rats, but the few rats per group and the conventionalization after weaning may have contributed to these results. In mature rats Combe (1973) found a greater weight gain with germfree rats even when the total gain was low due to the maturity of the animals. Also, the germfree rats gained 0.05 g more for each g diet eaten.

Levenson and Seifter (1974) reported unpublished data from experiments by F. Doft and E. G. McDaniel at the National Institutes of Health. These researchers were investigating low protein diets that caused liver damage and found that germfree rats grew faster than conventional rats on that diet. By adding the most limiting amino acids of the diet, threonine, tryptophan, and methionine to the basal low-protein diet, the conventional rats' growth rate improved so as to equal the growth rate of the germfree rats, which did not exhibit a response to the added amino acids. These results stimulated interest in the response to all ten essential amino acids. Germfree rats had at least a small advantage in growth when each of the essential amino acid, except valine, were deficient in the diet, but the greatest advantage was demonstrated when the diet was lacking tryptophan, arginine, or lysine. The

conclusion reached as to the reason for these results was that the intestinal microflora actually competed with the host for these amino acids at low protein intake levels. It was determined that in the rat the microbial destruction amounted to 40% of the methionine and 50% of the threonine and tryptophan in the basal diet. Therefore, if this destruction continues on a nitrogen free diet, the microbes are literally "robbing" the host of amino acids from the intestinal endogenous protein. The liberated nitrogen and sulfur could be used in microbial protein production, and the microbial protein excreted in the feces as part of the MFN. In somewhat the opposite type of experiment, Levenson and Seifter (1974) conducted experiments to determine the reason behind the more rapid death in starved germfree vs starved conventional rats. The ability to withstand starvation was not due to coprophagy in the conventional group. However, these researchers could find no reason for the decreased death rate. Salter (1973) explained similar results as being due to the cecal fermentation providing end products and/or microbial cells, presumably for nitrogen, to the animal. However, the decreased oxygen consumption, colonic temperature, and carbon dioxide production a few days before death point towards a decrease in the metabolic activities in the germfree animals. The key role of the B-complex vitamins in metabolism, and the lack of a fermentation to provide these vitamins when none were fed, may have led to the earlier deaths. Therefore, the deaths were probably not a direct effect of the cecal fermentation of nitrogen.

Chawla et al. (1976) fed graded levels of casein with and without neomycin, bacitracin, and polymyxin B sulfate. Except at one casein level without antibiotics, a linear response was observed from zero mg casein per g diet up to 135 to 202 mg casein per g diet. The most important observation was that the antibiotic supplemented rats were gaining 2 to 3 g per day more than their counterparts on the same protein level when antibiotics were not supplemented. With 10 to 11 rats per protein level, the average daily gain at zero protein with and without antibiotics was 0.46 and -1.96 g per day, respectively. However, upon further investigation, the data reveal enlarged ceca in the antibiotic supplemented rats, i.e., 1.09 g per 100 g body weight for rats without antibiotics, 2.20 g per 100 g body weight for rats with antibiotics. Therefore, the enlarged ceca tend to negate any body weight gain effect seen with germfree or antibiotic supplemented rats and mice as it occurs quite commonly in these animals (Visek, 1978; Reřat, 1978). Therefore, the best estimate of gain in body weight is the carcass weight change excluding the gastrointestinal tract weight. According to Coates (1973), enlarged ceca are unique to the rat and mouse. The increase in cecal weight and decrease in cecal microbes due to oral antibiotics is closely correlated (van der Waaij, 1969). Both reach their maximum and minimum, respectively, after 4 days in the mouse. Also discussed by Coates (1973) was the influence of kallikreins from the pancreas, saliva, or blood that will convert blood globulin to kallidin (a kinin) which reduces muscle tone in the cecal wall and allows its expansion. Schaedler (1973)

presented a similar concept except identified the kinin releasing agent as trypsin. With the modification of the cecal microflora, the kinin releasing agent is still active, while in the conventional animal it is destroyed by the microbial fermentation.

Wostmann et al. (1973) described the enlarged ceca of germfree and antibiotic fed rats as being due to high molecular weight mucopolysaccharides which are negatively charged. The enlarged ceca were found to reduce metabolic rate by 25%. Others (Gordon and Nakamura, 1975) proposed that the enlargement was due to an increase in colloid osmotic pressure which is similar to the Wostmann et al. (1973) proposal. El-Harith et al. (1977) made a similar proposal, but based their hypothesis on the presence of dietary raw potato starch in the cecum of conventional rats changing the colloid osmotic pressure. A somewhat opposing view is presented by Savage and Dubos (1968) who found a return of a bacterial population to the cecum after 30 days of antibiotic supplementation, but observed no decrease in cecal size. Therefore, it would appear that the resultant fermentation did not remove the factors implicated above.

In light of the above discussion, one advantage to using chickens for germfree and antibiotic modified animal investigations is the lack of the enlarged ceca. With germfree, conventional, and insolator raised conventional chicks, Forbes and Park (1959) found no growth response when feeding penicillin. The antibiotic may not have had an advantage in the conventional birds, but neither did it improve the

germfree birds' response. One could then surmise any antibiotic effect is not localized in the body outside the gastrointestinal tract. Similar results with germfree pigs (Whitehair and Thompson, 1956) and chick embryos in sterile conditions (Jukes and Williams, 1953) all point towards no antibiotic effect in the tissues of the host.

Miller (1967) found that from hatching to 21 days of age germfree chicks were 30 to 35 g heavier than conventional chicks. Growth rate increased in germfree chicks 10 to 20% compared to conventional chicks as reported by Riedel (1973). When feeding antibiotics there was no difference in growth rate observed between germfree chicks with and without supplementation. However, a toxin stress did reduce growth rate of the germfree chicks not supplemented with the antibiotics. Therefore, it was concluded that the antibotics had no effect in the absence of the intestinal microflora, unless the chick was subjected to a stress. This would be expected if the intestinal microflora functioned to detoxify any toxin taken into the gastrointestinal tract.

In an experiment in which no nitrogen was fed, other than from a dietary essential amino acid mixture, germfree chicks tended to gain about 0.4 g per day more than conventional chicks fed the same diet (Okumura et al., 1976). When urea was added to these diets conventional chicks significantly outgained the germfree animals fed the urea and the conventional chicks without urea. Germfree chicks fed urea gained less than those not fed urea. In fact, the depression in gain made this group's gain comparable to that of the conventional chicks without urea.

Therefore, it might be suggested that the extra stress on the animal to eliminate this additional nitrogen, from urea fed to the germfree chick or ammonia produced by protein-amino acid degradation in the conventional chick, may reduce gain.

Canadian workers (Miniats and Valli, 1973) used 126 pigs to study the effect of the microflora on the morphology and physical properties of the digestive tract. They obtained 96 pigs by hysterectomy, and half were maintained germfree. The others were purposely exposed to lactobacilli, streptococci, E. coli and Clostridium perfringens and were considered specific pathogen free (SPF) pigs. Both groups were raised in isolators. The remaining pigs were conventional pigs raised by their dam in a normal swine barn environment. This environment, in contrast to the isolator environment and diet of artificial milk, probably contributed to the two times greater gain of the conventional pigs vs either of the other groups. However, the germfree group tended to gain faster than the SPF pigs for 8 weeks. Klein et al. (1976) reported that carbadox improved nitrogen digestibility and digestible nitrogen retention by 3.0 and 14.2 percentage points, respectively. Although carbadox is not an antibiotic, but is a chemotherapeutic agent, it does modify the intestinal microbial population. Greatest improvement in gain and feed per gain was with the lower quality proteins. Lysine seemed to be the controlling factor, and the lower the lysine in the diet, the greater the advantage from carbadox. At the recommended level of carbadox, coliforms and Streptococci sp. numbers

were reduced 60 and 54%, respectively. Also indicated was a depression in metabolic activity of the bacteria. Reducing the protein level of a starter for 18 kg pigs was possible when lysine and carbadox are added to the diet (Veum et al., 1978). Carbadox, 0.17% lysine, and a 13% CP negative control diet produced gains and gain/feed ratios equal to the 16% CP positive control diet. Lysine or carbadox addition alone improved performance, but not to the extent of both together. Therefore, these studies indicate the number of bacteria and their activity were depressed, and lysine spared.

In general, the reports discussed above and the majority of those in the literature indicate a positive response to antibiotics. Additionally, germfree animals appear to grow faster than their conventional counterparts. This raises the question that if these results are extrapolated to zero nitrogen intake, where MFN is determined, will the effect be a reduction in fecal nitrogen excretion and additional growth?

Nitrogen excretion The only paper found in the literature that directly addresses the question of a reduction in fecal nitrogen excretion was published by a group of swine nutritionists at the University of Illinois (Harmon et al., 1968). The experimental animals were conventional and germfree rats fed either egg albumin or a casein hydrolysate at graded levels of each protein source. The trials were of 14 day duration with a seven day fecal and urine collection the last half of the trial. Feed intake was controlled by the rat in any initial weight outcome group that consumed the least amount. Because

rats on low protein diets will naturally decrease intake, this feeding procedure could have limited energy intake in the rats fed the higher protein diets. No data were provided as to actual intakes. The experimental purified diets were supplemented with egg albumin in experiments one and two to provide levels of CP of 0.42% (no albumin), 5%, 10%, and 15%. These diets were fed with and without autoclaving to conventional rats and only autoclaved to the germfree rats. In experiments three and four, a casein hydrolysate was added to diets as the protein source at 0.24% (no casein), 6%, 14%, and 22% CP levels. The diets were filtered through a millipore filter.

In the experiments where egg albumin was fed to conventional rats, the fecal nitrogen from the 0.42% CP diet averaged 1.47 and 2.01 mg per g DM consumed for the nonautoclaved and autoclaved diets, respectively. The germfree rats excreted 0.78 mg nitrogen per g DM consumed. Similar results can be concluded from data presented by Pecora (1953) in which the use of antibiotics decreased MFN from 1.23 to an average of 0.96 mg per g DM intake. Reddy et al. (1969) reported a greater nitrogen retention with germfree rats as compared to conventional rats. However, fecal nitrogen was higher with the germfree animals, but urinary nitrogen was decreased.

Recalculation of the reported extrapolated values of Harmon et al. (1968) at zero nitrogen intake based on data from all diets within each group resulted in fecal nitrogen values in mg per g DM intake of 1.41, 1.35, and 0.03 for the conventional nonautoclaved, conventional autoclaved, and germfree groups, respectively. Therefore, without the

microflora of the intestine to "rob" the animal of its intestinal endogenous nitrogen, there was very little MFN loss, about 2% as much as voided by conventional rats. The results presented could be due to the reabsorption and reutilization of the intestinal endogenous nitrogen on a low protein diet in the germfree rat. The one possible fallacy of this conclusion lies in the unknown intake of the rats. If intake was reduced severely with the low protein diets, this could have resulted in reduced energy intake and subsequently altered protein utilization and nitrogen excretion with higher protein diets. This concept of reabsorption and reutilization without intestinal bacteria does not agree with the findings of Loesche (1968), Combe (1973), and Okumura et al. (1976) in which case the bacteria were proposed to be needed to hydrolyze proteins and urea for required nonspecific nitrogen and to promote general well-being of the host (Schaedler, 1973).

In a report that indirectly supports the results of Harmon et al. (1968), and somewhat contradicts the statements made above, Pion et al. (1977) suggested that any influence of the microflora depends on the diet fed. Both conventional and germfree lambs were fed a high quality milk protein, while a heated casein diet was fed to both types of rats. With the lambs the nitrogen digestibility was decreased in the conventional, but the opposite was true in the rats. Using the average relative difference in amino acid composition, it was concluded that the germfree lambs excreted essentially no dietary nitrogen. However, with the conventional lambs bacteria contributed to the fecal

amino acid makeup. In the rats the fecal amino acid makeup was similar between the two groups and not very different from the ingested protein. Therefore, with a poorer quality protein more protein reaches the large intestine, and the microflora can increase its digestibility. However, with little dietary protein reaching the large intestine the microflora actually decreases apparent nitrogen digestibility. Antibiotic supplemented pigs fed 4 or 10% egg protein have been shown to retain greater quantities of nitrogen expressed as a percent of absorbed nitrogen (Francois, 1959). True absorption was unaffected indicating similar fecal nitrogen losses. While this result is somewhat in contrast to the research reported previously, nitrogen retention was improved.

As was discussed previously, the fecal nitrogen is composed of bacterial nitrogen, undigested dietary nitrogen, and intestinal endogenous nitrogen when a monogastric animal is fed a normal diet. The contribution of bacterial nitrogen to total fecal nitrogen has been reported to be as low as 50% (Levenson and Tennant, 1963) to as high as 97 to 100% (Mason, 1969) based on the ratio of 2,6-diaminopimelic acid in isolated bacteria and in a bacterial plus endogenous debris nitrogen fraction separated by detergent methods. Mason et al. (1976) also supplied data from pigs to show that the ratios of whole fecal amino acids to fecal bacterial amino acids indicated the fecal bacterial amino acids comprised 95 to 99% of the total amino acids voided. Even though the pigs were fed a diet containing soybean meal, the ratio

of the MFN's amino acids from two other laboratories to the whole fecal amino acids and fecal bacterial amino acids were 1.048 and 1.032, respectively. Although there may be differences in amino acid determinations between laboratories, this indicated that most of the fecal nitrogen, and more importantly, the MFN is due to bacterial nitrogen. Fecal ammonia production was decreased in conventional sheep, and therefore, much of the soluble protein in the cecum had been degraded before passing out in the feces (Hecker, 1971a). All these data tend to give credence to the concern of swine nutritionists as to the acceptability of fecal amino acids as an indicator of unavailable dietary amino acids.

Both fecal and cecal nitrogen data will be used to compare nitrogen losses of germfree animals to that occurring in conventional animals. Combe et al. (1965), Combe and Pion (1966), and a report of Combe's 1971 data by Refat (1978) describe a two- to fourfold increase in nitrogen in the cecum of germfree rats vs those from conventional rats regardless of dietary nitrogen level. Most of the nitrogenous compounds were in a soluble form in the germfree feces. The free amino acids in the germfree cecum were 50 to 100 times greater than the amount present in the conventional cecum and contained a high proportion of essential amino acids. Peptides of six to nine amino acid residues and mucoproteins were included in the soluble fraction. Levenson and Tennant (1963) demonstrated that $^{14}CO_2$ expiration was almost nil in germfree rats injected with ^{14}C-urea. Conventional rats, however,

expired large quantities of the ^{14}C, up to 2% in two hours of the total injected. Visek (1972) reported that urease has been found only in the digestive tract of most animals. Urease was an important generator of ammonia, and the ammonia was derived from mainly endogenous urea in normal situations. Hecker (1971b) and Nolan et al. (1976) demonstrated urease activity in the ileum, but the greatest activity was in the large intestine. Lack of urease in the experiments by Combe and Pion (1966) and in Combe's laboratory (1971) as reported by Refat (1978) allowed the urea concentration in the cecum of the germfree rat to approach that found in the blood, with only very small amounts of ammonia present. In contrast, the conventional rats had no urea in the cecum and up to 10 times more ammonia than their germfree counterparts. The insoluble fraction contained protein that had a similar amino acid makeup as the intestinal wall. This was found in the germfree rat to the greatest extent because this protein was exposed to the microbial fermentation in the conventional animal.

Also, intestinal enzymes could be degraded by the bacteria. Loesche (1968) measured the quantity of trypsin and chymotrypsin in the cecal contents of germfree and conventional rats. Expressed as mg per 100 g cecal contents, trypsin was 15 times and chymotrypsin 35 times higher in the germfree animal. Calculated as a percent of the insoluble nitrogen, trypsin's and chymotrypsin's contribution was 28 and 70 times higher in the germfree. The reason for the higher levels of amino acids, enzymes, and soluble and insoluble nitrogen was proposed to be a 90%

reabsorption of these intestinal endogenous nitrogen sources in the conventional animals. However, Loesche (1968) neglected any effect the intestinal microbes would have on this nitrogen. It is generally accepted that the free amino acid concentration in the rumen is quite low (Allison, 1970; Hecker, 1971b), and this suggests a rapid utilization (Chalupa, 1975) especially if the supply could be spread out over time. Also, soluble nitrogen tends to be easily degraded in the rumen (Hungate, 1966; Chalupa, 1975). Therefore, if the assumption is made that these types of nitrogenous materials in the gut of the germfree animal were also in the conventional animal's gut, then their presence could be masked by incorporation into bacterial protein and not really be an absorption into the host's blood system. Lepkovsky et al. (1966) indicated there was no difference in the total production of any protease, amylase, lipase, or trypsin in germfree or conventional rats. In the cecum and colon no difference in the activities of any of the enzymes was detected, except amylase which had a lower activity in the germfree rat. Whitt and DeMoss (1975) found a two- to threefold increase in free amino acids in the lower portion of the conventional mouse's small intestine. The lower concentration of free amino acids in the germfree animal was explained by a more rapid absorption or by a decrease in hydrolysis of intestinal endogenous proteins. In the cecum the total amino acid amount was higher, but the concentration was the same for germfree mice as compared to the conventional mice. Of the individual cecal amino acids found in greater concentrations, approximately equal numbers of essential and nonessential were present.

Salter (1973) indicated in the germfree chick the increased cecal urea and uric acid concentration increased their loss in the feces. Also mentioned was an increase in amino acids and peptides in the ceca of germfree chicks and a fivefold increase in ammonia in the conventional chicks. As with the germfree rat, soluble nitrogen constituted the largest portion of the total cecal nitrogen. Okumura et al. (1976) found somewhat different results in that the amounts of soluble nitrogen (amino acids, urea, ammonia) and insoluble nitrogen (proteins) were not different between germfree and conventional chicks. However, both groups of chicks were fed a purified diet plus the essential amino acids. This may have reduced endogenous secretions into the gut and tended to equalize nitrogen compounds moving into the ceca.

Henderickx and Decuypere (1973) found decreased urea and ammonia concentrations in the small intestine and cecum of antibiotic supplemented pigs. Vervaeke et al. (1976) found essentially no ammonia in the ileal contents of pigs fed carbadox. The decline in ammonia occurred concurrently with a decline in coliform and streptococci numbers. Using germfree and SPF piglets, Deguchi et al. (1978) found about a 10 fold decrease in cecal urea and a 40 fold increase in cecal ammonia in the SPF piglets fed urea as compared to the germfree animals fed the same diet.

Increased growth has been suggested to occur with a decreased urease activity (Harbers et al., 1963; Alvares et al., 1964). It was felt that the microbial fermentation would result in such a destruction

of exogenous and endogenous protein as to produce enough ammonia to be toxic to the intestinal cells and eventually cause death of the cells (Visek, 1972).

The total amount of nitrogen voided in the feces of the germfree and conventional animal has not been clearly defined. Reports can be found as to more or less nitrogen voided by the germfree animal. Coates et al. (1973) found a similarity in total nitrogen loss between the germfree and conventional chick fed normal diets. However, on a nitrogen free diet the germfree chick excreted more nitrogen, approximately 2 mg nitrogen per g diet eaten. Other reports from this same research group (Salter, 1973; Salter and Fulford, 1974; Okumura et al., 1976) suggest that at low levels of nitrogen feeding the germfree chick excretes more total nitrogen. However, they did state the differences were usually not significant and had no effect on total nitrogen excretion when protein was fed.

With the rat and the pig the opposite seems to occur. In germfree and antibiotic supplemented rats and pigs the fecal nitrogen was less than that voided by the conventional animal (Pecora, 1953; Harmon et al., 1968; Mason et al., 1976; Pion et al., 1977; Refat, 1978). Combe (1973) indicated a lower fecal and also urinary nitrogen loss in germfree rats. The nitrogen loss as a percent of intake was approximately the same with both groups. Levenson and Tennant (1963) showed data that indicated with increasing protein intake the germfree animal excreted more fecal nitrogen. However, total nitrogen excretion was

similar. No information was given as to the source of dietary protein. If the protein was of poor quality, it could have influenced the fecal nitrogen values reported.

With knowledge of the effect of the microflora in the rat and pig, it seems reasonable to assume the increase in excretion of nitrogen by the germfree bird may be due to the mixing of feces and urine in the cloaca. Also, due to the shortness of the large intestine and location of the ceca in the bird compared to the rat and pig, less reabsorption might take place.

In summary, it appears that on a low nitrogen diet or one containing a highly digestible protein, the germfree or antibiotic modified rat and pig excrete less nitrogen than conventional animals. In addition, there appears to be a growth response in these animals also. The nitrogen voided consists primarily of nonprotein nitrogen, mainly urea. Although developed for conventional chicks, a table presented by Salter (1973) tends to point out these effects and is presented in a modified form as Table 1. The table shows the effect of the intestinal microflora on nitrogen excretion and net protein utilization. However, it could also be used to explain effects in a germfree animal except where a microbial fermentation is required. For example, with the less than adequate protein level, amino acids can be released from intestinal endogenous protein, and the amino acids absorbed and utilized for protein synthesis. When this occurs, fecal nitrogen excretion decreases, urinary nitrogen excretion remains unchanged, and protein utilization increases.

Table 1. Possible effects on nitrogen excretion and net protein utilization values in monogastric animals[a] (Modified from Salter, 1973)

Action by intestinal microflora	Fate of N	Effect on Fecal N	Effect on Urinary N	Effect on NPU
1. Less than adequate dietary protein				
a. release of AA from protein	a. AA absorbed, not utilized	↓	↑	-
	b. AA absorbed, used for protein synthesis	↓	-	↑
b. deamination of AA	a. NH_3 absorbed, not utilized	↓	↑	-
	b. NH_3 absorbed, used for NEAA	↓	-	↑
c. incorporation of AA into microbial protein	a. less AA absorbed, and if from poor quality protein	↑	↓	↓,-
	b. less AA absorbed, and if from good quality protein	↑	-	↓
	c. less AA absorbed, alters AA balance	↑	↑	↓
2. More than adequate dietary protein				
a. release of AA from protein	a. AA absorbed	↓	↑	-
b. deamination of AA	a. NH_3 absorbed	↓	↑	-
c. incorporation of AA into microbial protein	a. less AA absorbed	↑	↓	-

[a]Nitrogen = N, amino acid = AA, non-essential amino acids = NEAA, net protein utilization = NPU, decrease = ↓, increase = ↑, no change = -.

For this series of events to occur, there must be some mechanism for reabsorption of the nitrogen in the germfree animal. If intestinal endogenous nitrogen is reabsorbed along with dietary nitrogen it would tend to balance out any amino acid deficiency in either as suggested by Refat et al. (1977) and could improve nitrogen utilization and growth.

Reutilization of intestinal nitrogen

In any consideration of reabsorption in germfree or antibiotic modified animals, it must be kept in mind that the microflora of the digestive tract populate and carry on a fermentation throughout the entire gut (Cranwell, 1968; Schaedler, 1973). The large intestine harbors the greatest number of bacteria (Refat, 1978), but when considering the nutritional effects of the absence or modification of the microbes, consideration must be given to the entire tract. Again, at this time, the ruminant will be excluded from the discussion.

With a germfree or antibiotic modified animal fed no nitrogen or a highly digestible protein, the intestinal endogenous protein must be spared in order to see decreased fecal nitrogen loss and/or increased growth. To have a sparing effect, the intestinal endogenous protein must be recycled, i.e., reabsorbed and reutilized. Fewer intestinal mucosa cells are shed into the lumen of germfree and antibiotic modified animals (Combe et al., 1965; Schaedler, 1973; Visek, 1978). Work by Snook and Meyer (1964) indicates that with diets similar to the zero egg or casein diets of Harmon et al. (1968) less intestinal endogenous

nitrogen would be present in the gut. Therefore, nitrogen quantities would be reduced somewhat in the gut as compared to when protein is fed.

It is well known that the small intestine has ample absorptive capabilities. Also, recent research by Ben-Ghedalia et al. (1976) has shown that challenging the ileum with protein when the animal is fed a low nitrogen diet can result in greater absorption than previously known. Therefore, it seems that the small intestine has ample time and capacity to reabsorb the intestinal endogenous nitrogen when low nitrogen diets were fed. Any intestinal endogenous nitrogen not degraded and absorbed in the small intestine would then be presented to the large intestine. Considering the increased transit time due to reduced peristalsis (Schaedler, 1973) and the enzymes being subjected to hydrolysis, one might expect a large quantity of free amino acids in the large intestine. Hecker (1971a) indicated sheep cecal liquor had a greater proteolytic activity than rumen liquor and that the proteolytic activity tended to increase rapidly above a pH of 6.0. The pH of the cecal liquor averaged 7.24. Since the pH optimum of trypsin is close to pH 8.0 (Mitchell, 1964), this would promote greater trypsin hydrolysis in the cecum. To be of benefit to the animal these liberated amino acids must then be absorbed from the lumen of the gut.

Kay and Pfeffer (1970) suggested 90% of the water passing into the large intestine was reabsorbed and that sodium absorption was more complete than that of water. Therefore, if free amino acids were

at a higher concentration in the cecum than the portal blood, movement with water would be expected. Ulyatt et al. (1975) noted that in sheep cecal contents the free amino acid concentration was 2.0 mg per 100 ml with the blood free amino acids concentration in the range of 1.8 to 3.6 mg per 100 ml. Therefore, only a twofold increase in free amino acid concentration would be required in germfree sheep to produce a concentration gradient toward the blood. According to Ulyatt et al. (1975), Demaux and coworkers in 1961, using a profused isolated sheep cecum, found that amino acids could be absorbed from the cecum. However, isolation of the intestine and its blood supply in this experiment could be considered to be unphysiological, and therefore, the results questioned.

Binder (1970) has shown that alanine can enter the colonic mucosa in vitro, basically due to diffusion. Using rats and ^{14}C-labelled E. coli, Yang et al. (1972) found that 30% of the label injected in the cecum could be found in the carcass. Of the label in the carcass, 20% was in a trichloroacetic acid insoluble fraction. Since carbon can become incorporated into almost any compound in the body, this experiment can not be used as conclusive evidence that the labelled amino acids as such were absorbed from the cecum and incorporated into body protein.

Slade et al. (1971) presented data indicating in the pony ^{15}N could be detected in the portal blood when bacteria labelled with ^{15}N was infused into the cecum. Wootton and Argenzio (1975) also suggested that amino acids could be absorbed from the large intestine of the

horse. However, Hintz et al. (1978) indicated that the absorption may not be as effective as previously thought. In the newborn piglet methionine has been shown to be absorbed in the colon (James and Smith, 1976). Also, after partial small intestine removal the colon was reported to take over some of the small intestine function. This assumption of small intestine function was proposed to be induced by the presence of digestive material in the colon.

Judson et al. (1975) injected ^{35}S-labelled ruminal bacteria into the cecum of sheep and detected nonreducible ^{35}S (organic form) in the plasma almost immediately. This nonreducible ^{35}S was proposed to be due to amino acid absorption. Only about 10% of the injected ^{35}S was retained, and that amount represented about 25% of that absorbed. Of the ^{35}S retained only 3% appeared in the wool. Elliott and Little (1977) infused $Na_2{}^{35}SO_4$ into the cecum to produce microbial ^{35}S-cyst(e)ine. However, none of the labelled cyst(e)ine appeared in the plasma, and it was concluded that the availability of the cecal microbial protein to the host was limited. The incorporation of the ^{35}S into bacterial protein in the cecum could have provided a more intact bacteria than that infused in the experiments of Judson et al. (1975). The intactness of the bacteria would explain the discrepancy between the results.

In agreement with the work of Elliott and Little (1977) are the data determined when proteins are infused into the cecum of an animal. Mason et al. (1977) infused graded levels of gelatin into the cecum of conventional sheep fed diets containing 13 to 15% CP. As the level

of gelatin increased there was a trend for fecal nitrogen, fecal 2,6-diaminopimelic acid, and fecal hydroxproline to increase, though none were significant. However, there were significant increases in urinary nitrogen, urinary urea, and blood urea with increasing gelatin infusion. Similar results with casein infusions into the cecum were found by Zebrowska et al. (1978b). Conventional pigs fed a 9.5% CP diet and infused with 120 g casein into the terminal ileum per day had an increase in fecal nitrogen as compared to animals given the casein orally. Also, urinary nitrogen increased, and therefore, resulted in a 30% decrease in nitrogen retention. Use of antibiotics did not change these results; however, the antibiotics did have an effect on the large intestine microflora as the digestibility of heated casein was reduced. In another experiment, using pigs fed a protein free diet, urinary urea nitrogen was increased with a cecally infused casein hydrolysate (Zebrowska, 1973). This increase led to the conclusion that the casein infused into the cecum was digested and absorbed, but then rapidly and completely excreted in the urine. These data, plus other data from the same laboratory, have resulted in Zebrowska (1978) concluding that there is only a small amount of amino acid absorption from the large intestine. In an earlier report Zebrowska and Buraczewski (1977) did report 2.5 and 3.0 g nitrogen apparently digested in the large intestine on a protein free and casein diet, respectively. Converted to a mg per g of DM intake basis, this quantity was approximately 1.5 to 2.0 mg per g DM intake. If this nitrogen was not utilized by the pig and excreted in the urine, but was added to the normal MFN

loss, MFN would then approach the amount observed in ruminants. Therefore, the MFN loss in ruminants may be slanted more towards the feces while in the pig the same quantity is lost via both feces and urine. It should be remembered that the diet of a ruminant contains more indigestible carbohydrate; this influence will be considered later.

It should be pointed out that the experiments reported above from Mason's and Zebrowska's laboratories were conducted with conventional animals. Dietary protein intake varied as did quantity and quality of protein infused into the large intestine. Also, all other research discussed in the consideration of large intestine absorption was done with conventional animals. Very little research has been reported on experiments specifically designed to investigate amino acid absorption in the large intestine of germfree animals. Kelleher and Bruckner (1977) demonstrated that the germfree animal was not less efficient as compared to a conventional in the absorption of alanine and glutamate in the ileum, colon, and cecum. Sodium was required for the absorption in all three segments, and therefore, the absorption appeared to be an active process. Therefore, the possibility of absorption in the lower intestine occurring can not be totally disregarded. In conventional animals fed normal diets few amino acids may reach the large intestine due to absorption and microbial degradation. If challenged with amino acids, when the host is fed a below adequate protein diet, the large intestine may respond as the ileum did in the research of Ben-Ghedalia et al. (1976).

Effect of Methionine-Microbial Interaction on Nitrogen Excretion

Although exact amino acid composition values for intestinal endogenous proteins have not been found, the data of Salter and Fulford (1974) with germfree chicks fed a nitrogen free diet suggest that methionine may be the most limiting amino acid. Mason et al. (1976) and Mason (1979) indicated an excess fecal excretion of methionine and cystine in relation to the other amino acids. Calculations of the fecal amino acid to bacterial amino acid ratio in pigs without antibiotics resulted in a value of 0.92 and 0.94 for methionine and cystine, respectively (Mason et al., 1976). The average for all amino acids was 0.99. With antibiotics the ratios became 0.75 and 0.78 while the average for all amino acids was 0.95. Therefore, it appears the bacteria in the gut tended to "tie up" the sulfur amino acids, and they were excreted. Francois (1959) indicated that the intestinal absorption of methionine and lysine was improved by antibiotic supplementation, thereby indicating less loss due to microbial fermentation. Mason (1979) using sheep found 10% more methionine and cystine in an isolate of fecal microbes than in the whole feces. Zebrowska et al. (1978a) described a net synthesis of methionine in the large intestine by measuring methionine entering from the ileum and leaving in the feces. Depending on the diet fed, there was a range from 10% disappearance to a 65% gain in methionine through the large intestine. Similar results were reported in an earlier paper (Zebrowska and Buraczewski, 1977). The most noticeable data reported in that paper were a -17% digestibility for methionine in the large intestine on a protein free

diet. Lysine's digestibility at +16% was the closest one to the methionine value. Total nitrogen digestibility was 57%. Salter and Fulford (1974) comparing the amino acid composition of germfree and conventional chick feces determined that methionine was also synthesized in the gut.

Naturally, the methionine synthesis was due to the microbial fermentation in the intestine. Moir (1979) stated that plasma sulfate could be recycled to the large intestine, and thus be a source of sulfur for microbial protein synthesis. The other source could be the intestinal endogenous protein (amino acids), especially on a protein free diet. If for any reason the microbial fermentation increases the degradation of the sulfur amino acids of the intestinal endogenous protein, changes in fecal and/or urinary nitrogen excretion might occur.

Effect of Fermentable Energy in Lower Intestine on Nitrogen Excretion

It is known that increasing the supply of digestible energy in the rumen will increase microbial protein synthesis if nitrogen is not limiting. Therefore, it would be expected that increasing the energy available for fermentation in the large intestine would result in greater nitrogen loss in the feces. In an indirect sense this would be evidence to support, but in an opposite manner, the conclusions drawn about nitrogen excretion comparisons between germfree and conventional animals.

Previously, the term indigestible carbohydrate has been used when considering diet components that affected fecal nitrogen excretion. More correctly, that term was used to describe the fermentability of the carbohydrate in the large intestine. In this section, the monogastric animal and ruminant will be considered separately as the ruminant normally consumes feeds having high levels of fermentable energy which could affect not only ruminal, but also large intestinal fermentation.

Monogastric animals

Mitchell (1926) stated that with diets low in indigestible carbohydrates the MFN would be approximately 2 mg per g DM consumed. However, the factor that had the most effect in increasing that figure was the level of indigestible carbohydrate. The indigestible carbohydrate was also implicated in the depression of apparent digestibility and in the increase in fecal nitrogen. No reason was given for the effects observed. A few years later, Mitchell (1934) fed high starch and high fat diets to rats. From the results of these experiments it was inferred that the indigestible carbohydrate effect on MFN was due to 1) increasing abrasion from the tissues of the lower gut and/or 2) a retardation of the absorption of the digestive secretions. These reasonings, especially the former, have been widely accepted as fact without giving due consideration to microbial fermentation activity.

Schneider (1934) developed a relationship and series of curves for MFN per unit of DM intake from the data of 1160 rats. Any increase

in the amount of indigestible carbohydrate would raise the average curve indicating an increase in MFN excretion, but would change the curve's form. Therefore, it was suggested that MFN determinations be made with diets of the same digestibility as the diets to be tested for biological value.

Increasing cellulose in the diets of rats had a detrimental effect on nitrogen balance (Duckworth and Godden, 1941). Total urinary nitrogen decreased, fecal nitrogen increased, and nitrogen retention decreased as cellulose increased. A similar experiment using increasing levels of cellulose in rat diets was conducted by Meyer (1956). A low protein diet was fed at a constant level with cellulose additions of 0%, 5%, 15%, or 30% in the diet. Increasing the cellulose increased fecal nitrogen per g intake at all levels, and the excretion on the 15% and 30% cellulose diets was significantly higher than on the zero cellulose diet. Endogenous urinary nitrogen was not affected by cellulose addition to the diet, but there was a trend towards a decline. If this trend was a true effect, it could have been due to a shift in the route of urea excretion from the urine to the feces.

Whiting and Bezeau (1957a) conducted an elaborate series of experiments to determine the effect of fiber on MFN excretion. Across all pig weights and CP levels additional cellulose increased the fecal nitrogen excretion. Urinary nitrogen decreased with added cellulose on the low protein diet, but was variable with the other protein levels. Correction of the apparently digested nitrogen for MFN excretion resulted

in similar true protein digestibilities. The MFN values for the 5%, 10%, and 20% cellulose diets were 1.02, 1.26, and 1.25 mg per g DM intake, respectively. There was no difference in the values determined by extrapolation and those determined directly. Therefore, it was suggested that MFN, for use in a biological evaluation, be determined at the same crude fiber level as the test diet. In a companion paper, Whiting and Bezeau (1957b) found that Solka floc was the source of indigestible carbohydrate that had the most effect in increasing the MFN loss as compared to oat hulls and methylcellulose.

Delort-Laval et al. (1968) fed either raw and cooked potato starch at 25 to 30% of the diet in protein free diets to pigs. The raw potato starch increased MFN losses by 60% and decreased apparent and true nitrogen digestibilities by 9 and 5 percentage points. However, nitrogen retention was not changed. Therefore, the increase in fecal nitrogen loss must have occurred at the expense of the urinary urea and therefore, reduced urinary nitrogen loss. This can also be inferred from the work of El-Harith et al. (1976) in which serum urea was decreased 9 mg per 100 ml when a 71% raw potato starch diet was fed in comparison to a 71% corn starch diet, both without any protein supplementation. Also, the maximum microbial nitrogen fermentation was found to occur with a 30% raw potato starch and 41% corn starch diet.

Even more dramatic increases in fecal nitrogen excretion were observed with potato starch diets by Mason and Palmer (1973). Rats were fed diets containing approximately 13.5% CP from egg albumin.

One experiment utilized adult rats; the other two used growing rats. Inclusion of raw potato starch at 10% or 22 to 25% of the diet increased the fecal nitrogen excretion 54 to 78% and 99 to 166%, respectively. This increase was in comparison to similar levels of corn, rice, and tapioca starches. The fecal nitrogen values from these control diets were quite similar to the MFN values discussed previously for rats fed lower levels of egg protein, and therefore, the egg contributed little to the fecal nitrogen. Presumably, these starches were almost completely digested in the small intestine, while the raw potato starch had physical properties of the granule that reduced its digestion in the small intestine but did not preclude microbial fermentation. The increased fermentation resulted in fecal nitrogen losses with the raw potato starch diets of 4.11, 3.46, and 4.69 mg per g DM intake in the three experiments, respectively. These were approximately twice the MFN values reported earlier for rats if one assumes no egg albumin contributed to these values. Age of the rats had no effect on this raw potato starch response. The greater effect observed as compared to that obtained by Delort-Laval et al. (1968) was probably due to excess egg amino acids being converted to urea, and urea diffusing into the large intestine and providing nitrogen for a greater fermentation. Yam starch and cellulose were also included in one experiment, and again fecal nitrogen was increased by 98% and 27%, respectively, at the 25% incorporation level. Therefore, raw potato starch had the most dramatic effect on increasing fecal nitrogen excretion.

In a later study, Mason et al. (1976) investigated the effect of unheated potato starch with and without antibiotics on fecal nitrogen excretion in pigs. The diets were 17.5% CP coming mainly from 12% soybean meal and 6% meat and bone meal with barley included at 54%. Therefore, with these natural feedstuffs in the diet fecal nitrogen will contain undigested dietary residues and not be a true measure of MFN. Starch, either potato or corn, was included at 25%. Neomycin sulfate and bacitracin were given at the rate of 1.75 g per pig per day. Using the detergent method developed earlier (Mason, 1969) on a centrifugally separated particulate fraction, the bacterial and endogenous debris nitrogen in the feces was determined. Also, a 2,6-diaminopimelic acid and RNA analysis were conducted on the feces. More than half of the nitrogen in the feces of the pigs not fed antibiotics was bacterial. Feces from animals fed antibiotics had approximately 30% of the nitrogen as bacterial nitrogen. Total nitrogen and α amino nitrogen (free and bound) voided in the feces were higher in the potato starch fed animals regardless of antibiotic supplementation. This increased loss could be a reflection of greater total nitrogen and α amino nitrogen passing the terminal ileum. However, calculation of the relative amounts of total nitrogen and α amino nitrogen between the corn and potato starch diets without antibiotics in the ileum and in the feces indicated an increase in fecal excretion of both fractions in animals fed the potato starch. The opposite effect was seen when similar calculations were compared with the diets containing antibiotics. Additionally, the percentage of the α amino nitrogen to total nitrogen

disappearing (absorbed) from the large intestine was similar in pigs fed no antibiotics regardless of starch source. With antibiotics the percentage of α amino nitrogen disappearing increased and accounted for 85% and 95% of the total nitrogen disappearing with corn starch and potato starch, respectively. It was concluded that the microbes were needed for a more complete utilization of the nitrogen in the gut. The data also indicated antibiotics spared α amino nitrogen for reutilization by the animal. Based on the amino acid composition of the digesta at the terminal ileum and in the feces, Mason et al. (1976) concluded that the nitrogen disappearing from the large intestine was of endogenous origin. The amino acid disappearance data also indicated that greater anounts of methionine were synthesized and excreted with the increase in cecal fermentation due to the potato starch. Including antibiotics in the diet resulted in an increased disappearance (absorption) of methionine in the large intestine with the greatest effect occurring with the corn starch diet.

One recent report indirectly disputes that fermentable energy has an effect on MFN. Eggum (1973) used cellulose in nitrogen free diets to determine the MFN value for each level of cellulose, but failed to report that value. At the same level of cellulose, casein plus 1% methionine was fed, and using the respective MFN value, true digestibility calculated. Because true digestibility did not change across 15 cellulose levels, the conclusion was made that fermentable energy did not affect MFN. This conclusion may be false due to the fact that with a highly utilizable protein source and the same level of cellulose the fecal

nitrogen voided would be the same on the nitrogen free and casein diets. Therefore, true digestibility would be biased toward being similar no matter the level of cellulose fed.

Ruminants

Consideration has already been given to the differing values for MFN between ruminants and monogastric animals. The value is approximately two to three times greater in the ruminant. Blaxter and Wood (1951) recognized this difference and found calves fed nonprotein liquid diets produced MFN values of somewhat less than normal, but still higher than monogastric animal values. They denied the suggestion that the large differences were due to a species difference, and instead proposed the differences to be due to the indigestibility of the DM. Due to the difficulties in developing a nitrogen and fiber free diet for ruminants, recycling of urea into the rumen, and the need for ruminal nitrogen for energy utilization, much of the research has not been designed to specifically investigate the fermentable energy effect on MFN. Therefore, many of the reports will be based on total nitrogen excretion.

Mukherjee and Kehar (1949), upon reviewing previous data, suggested that the indigestibility of the ration was closely related to, and more or less paralleled, the fecal nitrogen expressed per unit of intake. The quantity of indigestible components in the ration and the amount of fecal DM was implicated as an important factor influencing nitrogen output. In a companion paper, Kehar and Mukherjee (1949) compared the

MFN of cattle and rats fed diets similar in indigestible energy content. When feeding low nitrogen diets with increasing crude fiber to either cattle or rats, the fecal nitrogen expressed as mg per g DM consumed increased as has been reported previously in this discussion. However, when the fecal nitrogen was expressed as mg per g of fecal DM the values decreased. More importantly, the coefficients of variations of the data were smaller for the values expressed on a per unit of fecal DM. Therefore, not only did fermentable energy in the large intestine increase fecal nitrogen, but expressing fecal nitrogen on a fecal DM basis resulted in a more consistent figure, especially at the higher levels of indigestible carbohydrate. Inference can then be made that MFN is more closely related to indigestible matter or fermentable energy in the large intestine.

Using sheep fitted with a re-entrant cannula near the ileo-cecal junction, Thornton et al. (1970) demonstrated a significant effect of fermentable energy in the cecum on nitrogen excretion. Mature wethers were fed a diet of oat hulls and alfalfa containing 7.0 to 7.5% CP, which would be a little below their maintenance requirement for protein. Into the cannula either 0, 30, 60, or 90 g of glucose were infused each 24 hours. The results can best be presented in Figure 1. The cecal glucose supplementation significantly increased fecal nitrogen, urinary nitrogen decreased to the same extent, and nitrogen intake remained constant. With the changes in nitrogen excretion equalized, nitrogen balance remained constant. The decrease in urinary nitrogen was

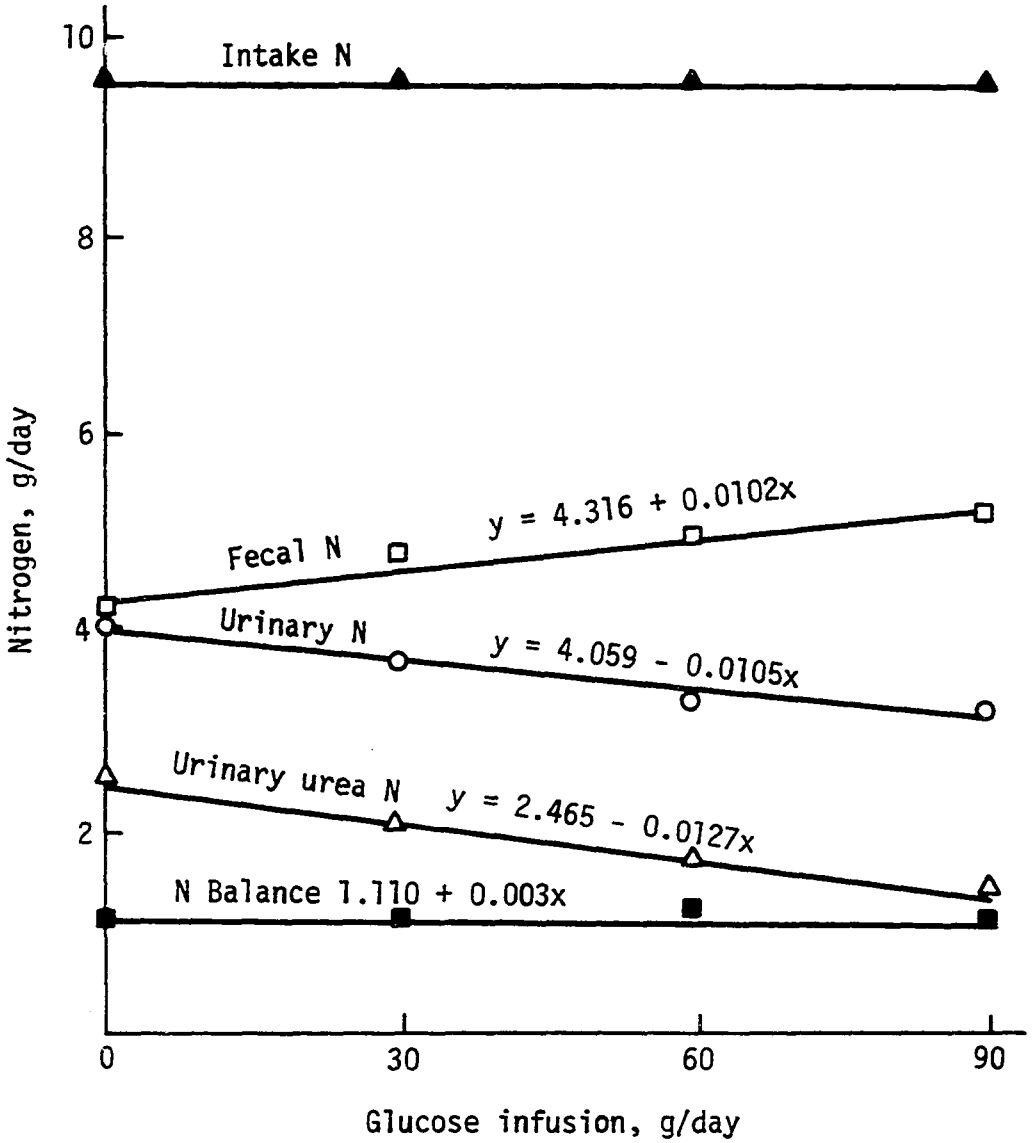

Figure 1. Effect of cecal glucose supplementation on nitrogen intake, excretion and balance (Thornton et al., 1970)

paralleled by a significant decrease in urinary urea excretion. Increases in fecal nitrogen could not be accounted for by the nitrogen passing through the ileum, and therefore, had to come from blood urea. Comparison of the slopes of the regression lines for fecal nitrogen and urinary urea indicated urinary urea declined at nearly the same rate that fecal nitrogen increased. Therefore, it can be assumed that the urea was covering the nitrogen requirement of the increased fermentation, and it was not being met by intestinal endogenous nitrogen. Thornton et al. (1970) suggest that transfer of urea to the rumen or large intestine is the preferential pathway for urea excretion in ruminants when fermentation rates are high in either area.

Ørskov et al. (1970) conducted a similar experiment in which corn starch was infused into the cecum. The starch increased fecal nitrogen excretion three to four g per day or about 60% with two sheep. The increase was almost equally distributed between the bacterial and endogenous debris nitrogen and water soluble nitrogen fractions. The bacterial and endogenous debris nitrogen was composed of over 70% of particulate bacterial debris and small amounts of epithelial cells and food residues. Enzymes residues were the primary constituent of the water soluble nitrogen (Mason, 1971). In another experiment using only one sheep, fecal nitrogen was not increased with oral feeding of starch, but a similar amount infused into the cecum did increase fecal nitrogen (Ørskov et al., 1970).

Pectin and starch (presumably corn) were shown to be more effective than cellulose in increasing fecal nitrogen excretions when infused into the cecum (Mason et al., 1977). The diets were predominantly grass hay and contained approximately 14% CP. The fecal nitrogen increases were accompanied by greater quantities of bacterial debris in the feces and by a decline in urinary nitrogen. Starch infusion resulted in fecal nitrogen increases up to an infusion rate of 110 g per day. Ruminal bacterial residues were surely a part of the fecal nitrogen, but were assumed to be similar on all treatments. This should have created no problem as far as total fecal nitrogen is concerned. However, estimates of microbial nitrogen synthesis in the large intestine could have been biased upward due to the presence of ruminal bacterial residues.

Mason (1979) investigated the effect of ruminal bacteria residues on fecal bacterial residues using data from 47 digestibility trials. The majority of the diets could be characterized as containing medium to good quality forages with higher levels of CP content (>9% CP). A few high concentrate diets were included. All diets were fed to sheep. Bacterial and endogenous debris nitrogen excretion was found to be related to the intake of digestible nitrogen. On these "normal" diets bacteria made up a major portion of nitrogen loss via the feces. The relative contribution of the ruminal and cecal bacteria would depend on the fermentation at each site and on any factor that modifies either fermentation. Characterization of the diets into roughage and

concentrate diets leads to the conclusion that the increase in fecal bacterial and endogenous debris nitrogen with concentrate diets was due to more endogenous nitrogen because the 2,6-diaminopimelic acid concentration was decreased in the feces. There was some logic to this assumption because the rate of passage should be higher with the concentrate diets, and therefore, would reduce retention time in the large intestine. But in contrast, different amounts of fermentable energy can result in differing microbial protein production (Burroughs et al., 1975). Therefore, much variation can be encountered in fecal nitrogen excretion in ruminants.

Some of these variations were detailed in a review of factors affecting protein requirements for maintenance in cattle (Swanson, 1977). By using data from 16 different experiments, it was concluded that MFN losses were more closely related to fecal output than DM input. The data used were from experiments in which semi-purified diets were used. Therefore, this conclusion fits well with previous conclusions presented in this Literature Review. In another comparison data from 70 low protein forage diets were used. Essentially no difference was found when fecal output was expressed per DM intake or per fecal DM. Detailed descriptions of different diet effects were given, but basically it was pointed out that the fecal nitrogen output in ruminants can be quite variable mainly due to the fermentation in the rumen. However, this fermentation is a necessity in the ruminating animal for energy and protein. If energy and protein are not provided,

the animal's physiology begins to change, and this can alter fecal nitrogen excretion.

Effect of Practical Rations on Nitrogen Excretion

Most of the previous sections of this review have centered around the monogastric animal fed semi-purified diets of low protein content. Some reports of monogastric animals fed more normal diets were mentioned, while very little of the ruminant data has been with purified diets of lower protein content. The following discussion deals with the effect of higher protein intakes in normal diets as would be encountered in practical situations. With the pig this would be basically higher starch diets and with ruminants higher fiber diets, except those fed in feedlots. Therefore, increased starch digestibility and increased rate of passage could present less fermentable energy (starch) to the large intestine of both the feedlot ruminant and swine. Higher intakes of forages by ruminants and some swine, such as the sow, would decrease rate of passage in, present more fermentable energy to, and prolong the fermentation of that energy in the large intestine. Although affected by many factors, the following discussion assumes this fermentable energy presented to the large intestine more than meets the microbial energy requirement.

Therefore, nitrogen would be limiting, and as discussed previously, the microbes can degrade intestinal endogenous protein for their nitrogen if it is the only source available. However, on a practical ration, this should not be the case. Data by Ford and Milligan (1970)

indicate that urinary urea excretion is proportional to plasma urea and that urea recycled to the gut, as measured by difference, is also proportional to plasma urea. Similar results were found by Hecker (1971b) in which the ileal contents contained urea at a concentration approaching the blood level. Because very little urea could be detected in the cecum, blood urea nitrogen and cecal ammonia nitrogen were found to be closely related. Plotting ileal urea nitrogen and cecal ammonia nitrogen vs blood urea nitrogen results in two essentially parallel lines with cecal ammonia nitrogen approximately twice the ileal urea nitrogen. Ileal nitrogen flow into the cecum accounted for 79% of the cecal ammonia nitrogen. These results are similar to the data of Thornton et al. (1970) in which increased large intestinal fermentation pulls urea from the urine, and the nitrogen is then excreted in the feces as microbial protein. In a companion paper, Thornton (1970) demonstrated nitrogen accumulation in the rumen to be similar at variable plasma urea concentrations up to 15 to 17 mg per 100 ml. Although not demonstrated, one might expect this same relationship to exist within the large intestine.

Cocimano and Leng (1967) indicated that sheep fed a 3 to 4% CP diet excreted in the urine only 10 to 15% of the urea produced, the remainder being degraded in the intestine. As CP intake increased, more urea, as a percentage of total urea production, was lost via the urine, but increasing quantities were also degraded in the gut. These data represented degradation in the entire gut and not just the large intestine.

In subsequent papers from this same laboratory (Nolan and Leng, 1972; Nolan et al., 1973; Nolan et al., 1976; Mazanov and Nolan, 1976)

an attempt was made to more specifically quantitate the amount and site of this degradation. From these data it was estimated that 75 to 80% of the blood urea degraded in the total intestine occurred outside of the rumen. The cecum accounted for 20%, and it was suggested the remainder was degraded in the ileum or large intestine posterior to the cecum. Microbial fermentations do occur in these regions (Cranwell, 1968; Schaedler, 1973), so it is logical to expect urea degradation to occur. The diets fed were high in nitrogen being about 18 to 20% CP.

Therefore, it can be concluded that large quantities of urea are degraded in the intestine. As protein intake increases it can be demonstrated that urine nitrogen increases (Harmon et al., 1968; Bunce and King, 1969a,b). As protein intake increases blood urea also tends to increase, and a rapid increase occurs as protein absorption exceeds that needed for utilization of body processes. Based on the data by Ford and Milligan (1970) and Thornton et al. (1970), this increase in urinary urea is due to an increase of blood urea. The concentration at which urea entry into the gut declines, 15 to 17 mg urea per 100 ml (Thornton, 1970), would be considered to be on the high side of normal (Swenson, 1970). Therefore, the level of blood urea would probably never exceed the gut's capacity for urea diffusion, and urea would be constantly supplied to the fermentation sites in the gut.

Again only considering the monogastric animal, this urea could supply the microbes requirement for nitrogen as Bryant and Robinson

(1961), Hungate (1966), Allison (1970), and Chalupa (1972) reported a usage of ammonia by rumen microbes for microbial protein synthesis even in the presence of preformed α amino nitrogen. Because this is nitrogen that would have been lost via the urine, but is now lost in the feces, the question arises as whether or not intestinal endogenous nitrogen degradation is reduced. If so, then any reabsorption and reutilization of these amino acids should improve nitrogen balance. If it is assumed that this reutilization is essentially complete at all levels of protein intake, then at zero nitrogen intake nitrogen balance should also approach zero. Based on data available in the literature, extrapolation of nitrogen balance data at higher protein intake can be found to approach the origin of a graph. Admittedly, there may be some bias introduced by the points chosen and by the few number of points used in some cases. Only a few examples are presented; however, similar relationships can be found in other reports. Also, similar relationships can be found by replacing nitrogen balance with body weight gain in experiments with rapidly growing animals. Only nitrogen balance experiments will be considered in this discussion. For simplicity, graphs of these types of data are presented in Figures 2 to 6. Open symbols represent points of nitrogen intake ignored in the development of the regression equations. Data presented by Black and Griffiths (1975) from approximately 300 calves fed liquid diets varying in nitrogen content could not be presented in a similar fashion to the other data. However, extrapolation of their graphic data also results in similar results to those presented by others.

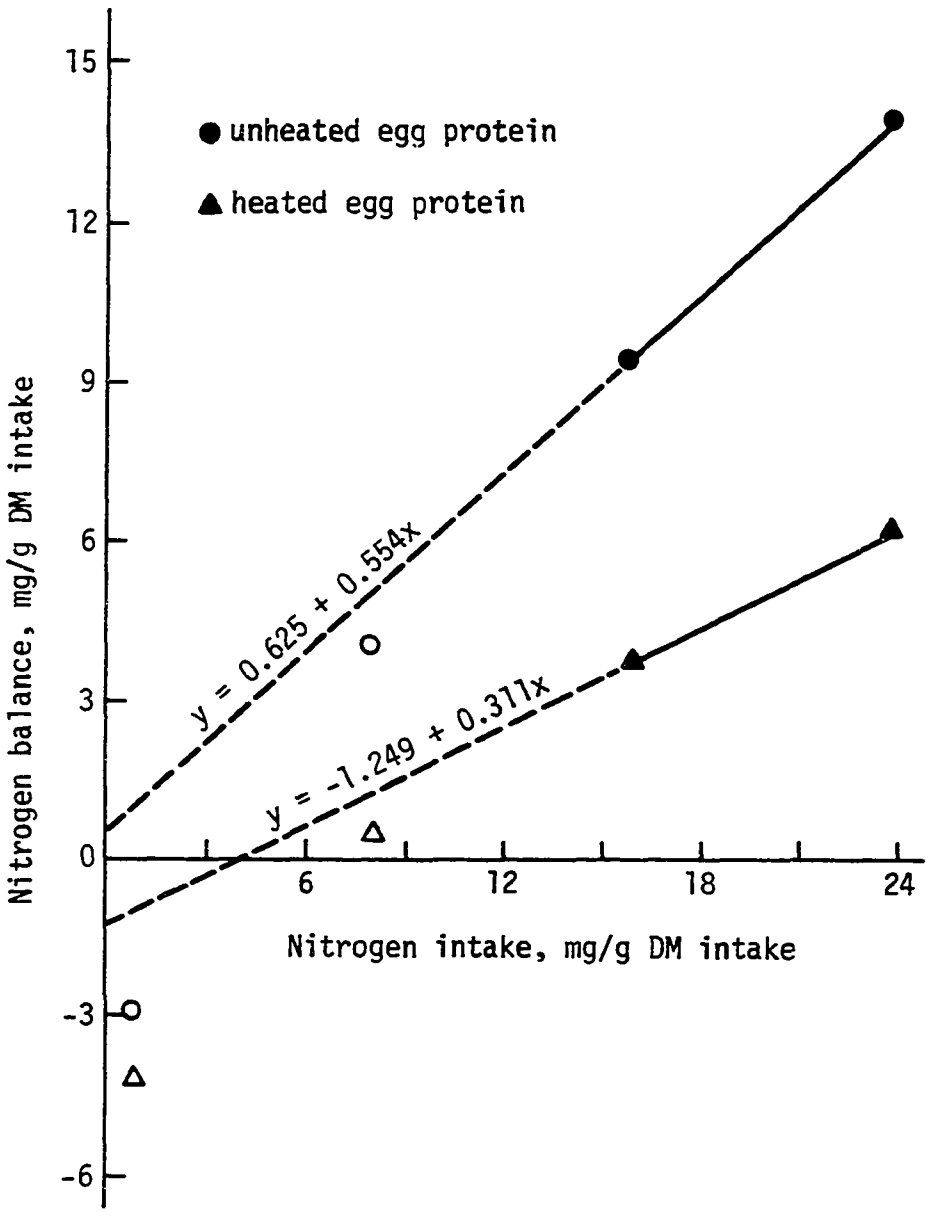

Figure 2. Relationship of nitrogen intake to nitrogen balance in rats fed egg protein (Harmon et al., 1968)

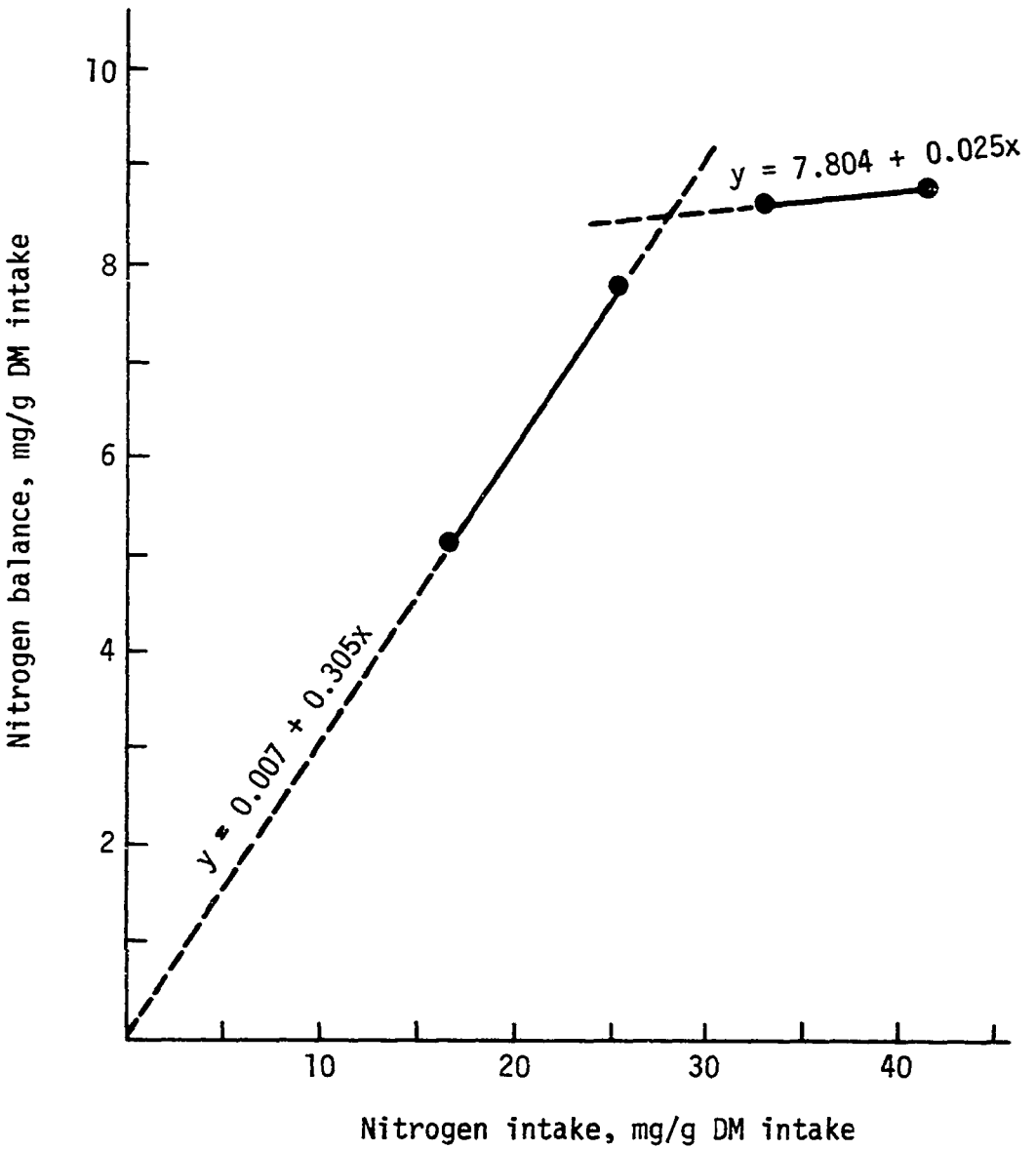

Figure 3. Relationship of nitrogen intake to nitrogen balance in rats fed casein (Forbes et al., 1935)

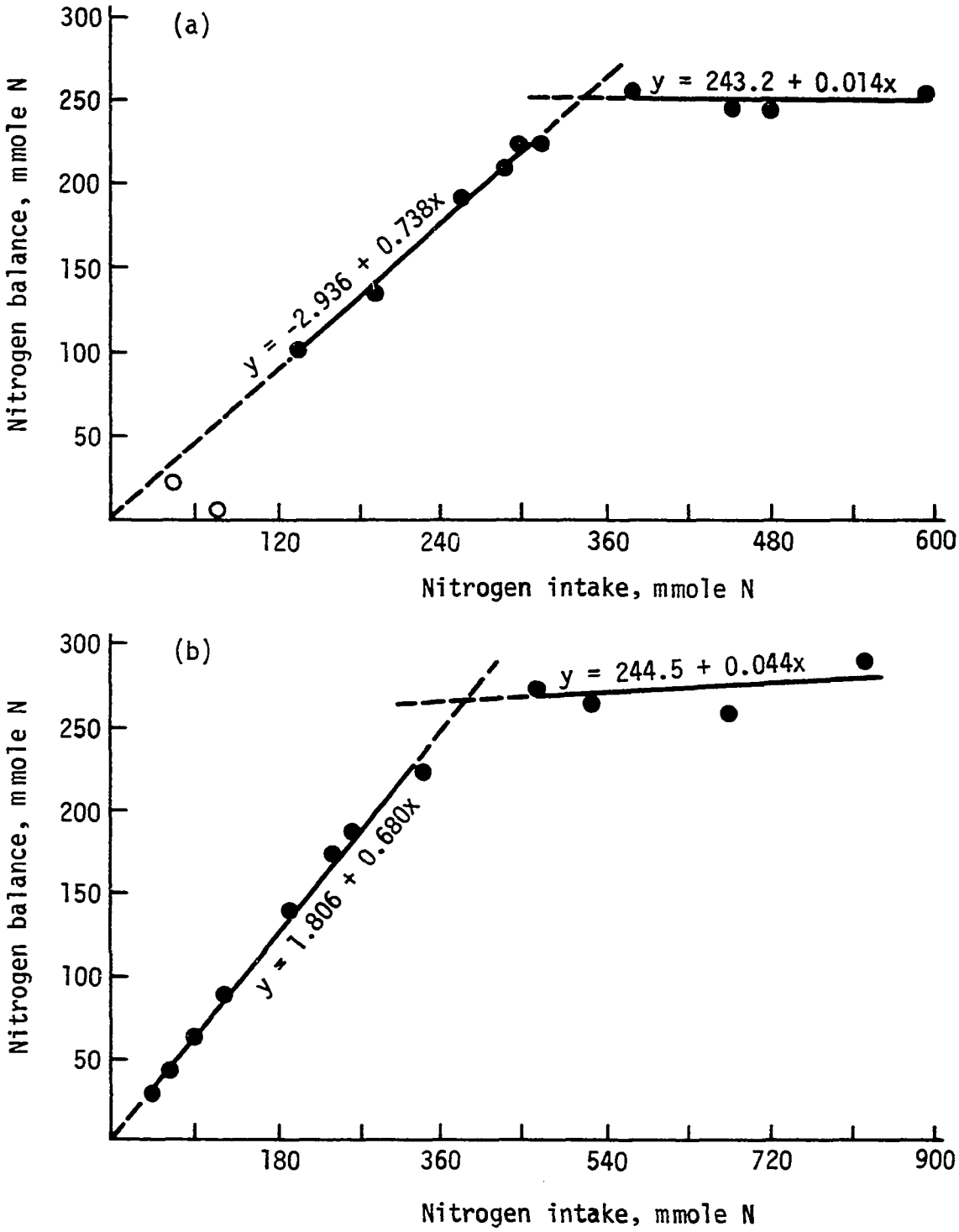

Figure 4. Relationship of nitrogen intake to nitrogen balance with: a) lactalbumin, b) casein (Bunce and King, 1969a,b)

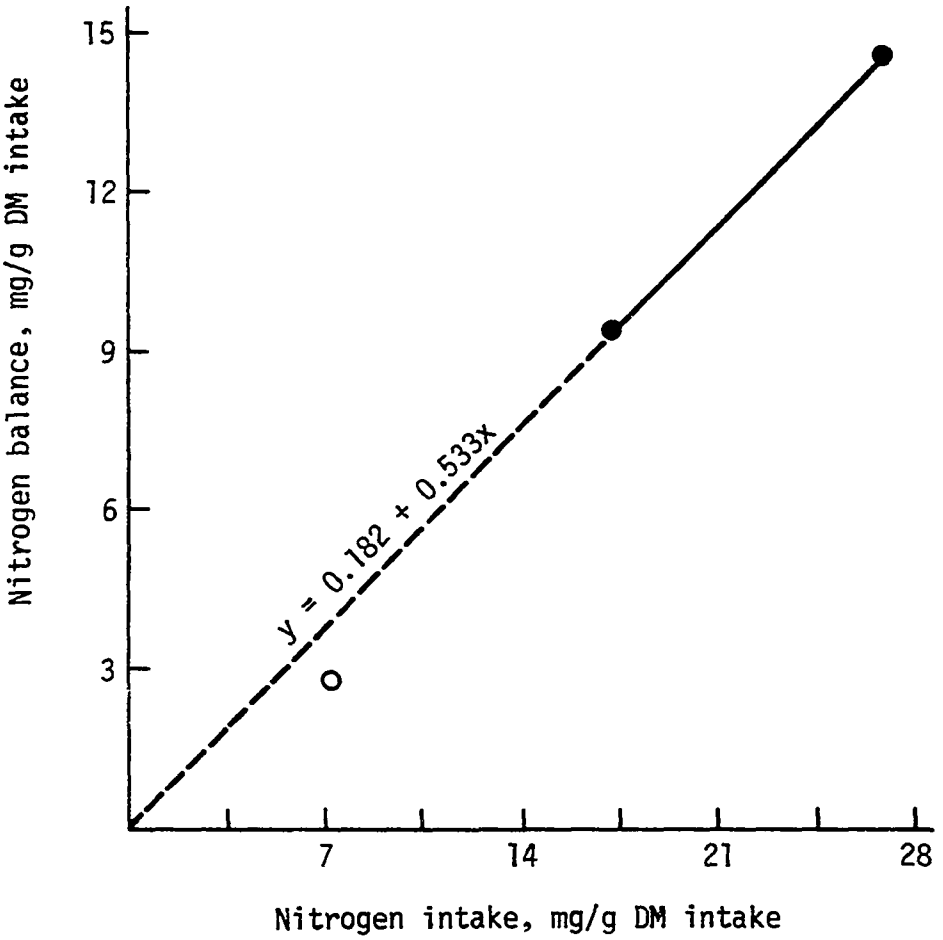

Figure 5. Relationship of nitrogen intake to nitrogen balance in pigs fed soybean meal (Armstrong and Mitchell, 1955)

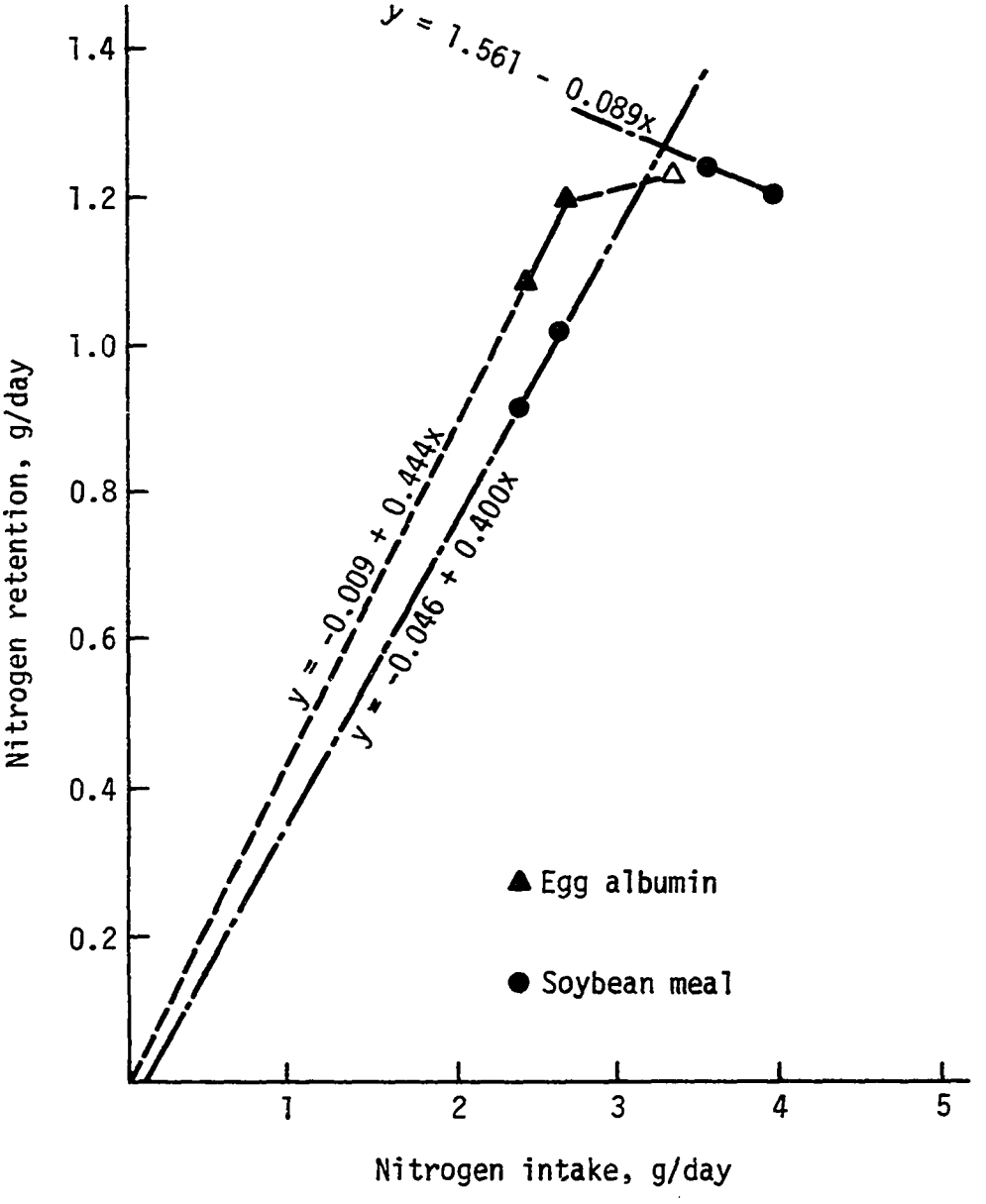

Figure 6. Relationship of nitrogen intake to nitrogen retention in laying hens with two protein sources (Shapiro, 1968)

The data presented do include experiments which resulted in data points at low protein intakes where less nitrogen retention occurred than predicted by the equations (Armstrong and Mitchell, 1955; Harmon et al., 1968; Bunce and King, 1969a). Using extrapolations to zero nitrogen intake, there seems to be an indication that nitrogen balance approaches zero at zero nitrogen intake. It is known that this can not be true due to many losses of nitrogen that can not be recovered. Such losses from hair, wool, feathers, hoofs, indole, 3-methylhistidine, and ammonia for acid base balance are irreversibly lost from the animal. However, the total quantity of nitrogen lost from the body via these pathways may be small and difficult to measure during short periods of time.

EXPERIMENTAL PROCEDURES

Rat Growth Trials

Sprague-Dawley male rats, initially weighing 40 to 50 g, were maintained during the trials in a small animal laboratory. Rats were purchased at this weight in order to provide a more uniform group at the starting weight of approximately 120 g. The lighting pattern was a 12-hour cycle with light from approximately 0600 to 1800 hours. Temperature was maintained at approximately 24°C.

Upon arrival, two rats were placed in each metabolism cage (Holtage, Model 45238) without the excrement collection apparatus and with the tunnel feeder blocked. For approximately one week the rats were fed a commerical laboratory chow ad libitum. After that time, they were fed ad libitum a purified diet using stainless steel feeding cups (trial 1) or the tunnel feeders (trial 2, 3, and 4). The cages were placed in a conventional rat cage rack that would normally hold 30 cages per side in five rows. This allowed for 32 cages to be alternated with an empty space between each (except for 2 cages on each side) so an estimation of any feed wastage could be made without contamination from adjacent cages. In the last trial, four rats were housed on another rack in conventional cages and fed in stainless steel cups those diets that normally presented no feed wastage problems.

At approximately 120 g, all rats were weighed, stratified by weight into three outcome groups, and randomly assigned to an individual cage. Previously the cages had been blocked on vertical location in the rack, and each diet randomly assigned within a block. The composition of the diets is presented in Table 2. The 21.00% CP diets were fed during the first three trials and then replaced by the 7.88% CP diet during the last trial. Water was added to make all diets approximately 90% DM on an as-fed basis. The composition of the antibiotic premix added to the diets and the quantities mixed in the drinking water are shown in Table 3. Antibiotics and levels were similar to those of Chawla et al. (1976). The purified adjustment diet contained either 7.88 or 10.50% CP depending on the trial and contained no antibiotics.

The first and last trial were initiated with 36 rats; three per diet. The other trials began with 32 rats; three rats per diet except the 5.25% and 10.50% CP diets with and without antibiotics which had two rats per diet. The number of rats in these trials was limited by the number of metabolism cages available. Therefore, including the three initial slaughter rats per trial, a total of 148 rats was originally started on the diets. Due to problems discussed below, the trials ended with unequal numbers per treatment. Actual numbers will be presented in tables in the Results and Discussion section.

Rats were weighed daily, and based on that weight, fed 1.8 times the NRC (1972) energy requirement for maintenance. In the first trial diets were administered as a slurry with a curved, 16 gauge, 3 inch,

Table 2. Percentage dry matter composition of diets fed to rats during growth trials[a]

Calc. crude protein, %	0.00	2.62	5.25	7.88	10.50	15.75
Albumin, Egg[b]	0.00	3.0	6.0	9.1	12.1	18.1
Antibiotic Premix[c]	--	--	--	--	--	--
Corn Starch	58.0	58.0	58.0	58.0	58.0	58.0
Dextrose	25.7	22.7	19.7	16.6	13.6	7.6
Sucrose	5.2	5.2	5.2	5.2	5.2	5.2
Butter	5.1	5.1	5.1	5.1	5.1	5.1
Salt, R.H.[d]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Premix[e]	1.0	1.0	1.0	1.0	1.0	1.0

[a]All diets formulated on a DM basis to contain 411 kcal/100 g DM.

[b]ICN, Nutritional Biochemical Division, Albumin (egg), #100370.

[c]Antibiotic premix composition: Table 3.

[d]ICN, Nutritional Biochemical Division, Rogers and Harper salt mixture, #902842.

[e]Vitamin premix composition: Table 4.

21.00	0.00	2.62	5.25	7.88	10.50	15.75	21.00
24.1	0.0	3.0	6.0	9.1	12.1	18.1	24.1
--	1.0	1.0	1.0	1.0	1.0	1.0	1.0
58.0	58.0	58.0	58.0	58.0	58.0	58.0	58.0
1.6	25.3	22.3	19.3	16.2	13.2	7.2	1.2
5.2	4.4	4.4	4.4	4.4	4.4	4.4	4.4
5.1	5.3	5.3	5.3	5.3	5.3	5.3	5.3
5.0	5.0	5.0	5.0	5.0	5.0	5.3	5.3
1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 3. Antibiotic premix and drinking water mixture for rats during growth trials

	Diet Premix[a] %	Water Mixture g/10 l
Neomycin sulfate[b]	11.04	13.0
Bacitracin[c]	10.37	5.6
Polymyxin B sulfate[d]	1.1	.6
Sucrose	77.49	--

[a]Included at 1% of diet.

[b]Sigma Chemical Co., #N1876.

[c]Sigma Chemical Co., #B0125.

[d]Sigma Chemical Co., #P1004.

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[c]Sigma Chemical Co., #B0125.

[d]Sigma Chemical Co., #P1004.

Table 4. Vitamin premix used in diets for rats during growth trials[a]

	g
Biotin[b]	0.039
Calcium pantothenate	1.2
Choline chloride	60.0
Folic acid	0.06
Inositol	3.0
Menadione	0.15
Niacin	1.2
Para-amino benzoic acid	3.0
Pyridoxine HCl	0.15
Riboflavin	0.24
Thiamine HCl	0.15
Vitamin A powder (20,000 IU/g)	30.0
Vitamin B_{12} premix	0.9
Vitamin D_3 (400,000 IU/g)	0.15
Vitamin E (250 IU/g)	12.0
Sucrose	187.8

[a]Formulated to meet A.O.A.C. (1965) requirements when used at 1% of diet.

[b]Biotin increased to meet NRC (1972) recommendation when feeding egg white.

stainless steel feeding needle (Popper and Sons, Inc., New York, #7916). Intubations were carried out by holding the rat in a vertical position in the left hand and inserting the needle attached to a filled 10 ml syringe with the right hand. The needle was passed along the palate until it reached the approximate location of the pharynx. Normally the rat would exhibit a gagging reflex at which time the needle would be quickly, but carefully, passed into the esophagus. By use of a radiograph it was determined the needle was approximately three-fourths the length of the esophagus before the diet was expelled from the syringe. The diets were made into a slurry by mixing the dry diet plus water (1:1 w/v) with a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY), and then subjecting the slurry to a vacuum to remove as many air bubbles as possible. In one series of preliminary trials failure to remove the air bubbles resulted in death of the rats due to gastric tympany (bloat) (ISU Veterinary Diagnostic Laboratory Case Numbers 10461 and 10612). DM content of the slurry diets was estimated based on the exact mixture of diet and water used and on a DM analysis of similar mixtures in the preliminary trials. This provided the basis for the volume of diet to be intubated. Exact intakes were then calculated after the completion of the trial based on analysis of each slurry mixture prepared. In the first trial intubations were necessary at eight hour intervals in order to provide the required daily DM intake. Due to the great number of intubations required with this feeding schedule, accidental drowning of the rats became a problem.

At the end of the tenth day this trial was terminated so as not to further reduce the number of rats per treatment. In subsequent trials the tunnel feeders that attached to the metabolism cages were used, and the rats allowed to consume as much dry feed as possible. If the required amount of feed was not consumed by 0800 hours the following day, the rat was intubated with an amount equivalent to that not consumed. Depending on volume of slurry needed and rat weight, the slurry was administered in as many as three intubations over a 12 to 14 hour period. In general, the lowest and highest protein diets required the most intubations. This modified feeding scheme reduced the loss of rats caused by drowning, and the remaining trials were carried out for 14 days.

Sample Collection

Diets were mixed in adequate amounts to last an entire trial, and therefore, one sample of each diet was taken and kept at -18°C. Equal aliquots of individual slurry diets were composited each time a new mixture was prepared and stored at -18°C.

Initially, collection of feces and urine were planned for nitrogen balance determination. However, diarrhea with rats intubated and/or fed antibiotics prevented this collection.

Analysis of carcass nitrogen was carried out, and nitrogen balance calculated by difference between nitrogen content at the beginning and end of each trial. At the start of each trial, one rat from each

outcome group was sacrificed to determine an initial level of carcass nitrogen. Rats were anethesized with approximately 0.3 to 0.4 ml of a 2.5% solution of sodium thiopental, and then their diaphragm severed. This procedure prevented blood loss, and the blood contributed to the total carcass nitrogen content. In order to prevent errors due to feed remaining in the digestive tract, the intestines were removed by severing the esophagus at the diaphragm and the rectum at the anus. Gastrocnemius muscle from both legs was removed, weighed, and frozen immediately. The liver was removed and weighed, placed back with the carcass, and the two weighed together. The carcasses were frozen at -18°C. For all calculations involving carcass weight, the gastrocnemius muscle weights were added back to the carcass weight. Carcass weight in this study refers to body weight minus the weight of the gastrointestinal tract and its contents.

Blood samples were collected simultaneously from the aorta and portal vein of each rat. The difference between nitrogen components of the portal and aorta sample provided a measure of intestinal uptake or output. In order that all animals would be in approximately the same absorptive state, feed was removed for two hours and then replaced for two hours before sampling except in the first trial. Due to the length of time required to collect the samples in the first trial, the rats were not all in the same postabsorptive state. Rats that normally did not consume adequate dry feed were intubated two hours before being sampled. Animals were selected at random from within each block for

sampling, so any time effects were spread equally across all treatments. Anesthesia was provided by approximately 0.3 to 0.4 ml of a 2.5% solution of sodium thiopental. Using heparinized syringes, 1.02 mm plastic tubing, and a 26 gauge, 9.5 mm needle, approximately 0.4 ml of blood was taken from the aorta and portal vein simultaneously. The blood was diluted 1:10 (v/v) with distilled water with an automatic dilutor (Micromedic Model 25004), and the diluted sample frozen at -18°C, and stored until analyzed.

In addition to the above samples, the cecum was removed from each animal completing the experiment by cutting the small intestine and colon approximately 2 to 4 cm from the cecum. The cecum was then weighed with very little loss of contents. Except in the first trial, an incision was then made in the cecum and a sample of the cecal contents taken and frozen. Cecal samples were stored at -18°C. The cecum was then washed out with distilled water, blotted as dry as possible, and the washed cecal tissue weighed. Weight of cecal contents was determined by difference.

Laboratory Analyses

Diets were analyzed for nitrogen by the Kjeldahl method (A.O.A.C., 1965) on an as-fed basis. Samples of the slurry diets were analyzed for DM content by freeze drying. Diet DM was also determined by freeze drying and used to convert slurry DM intake to as-fed intake. All diet samples were analyzed in triplicate.

Carcasses were prepared for analysis by grinding in liquid nitrogen using a commercial Waring Blendor (Model 91-186). Prior to grinding, the frozen carcasses were cut into several pieces to facilitate grinding. As soon as the liquid nitrogen evaporated, the samples were again placed in -18°C storage. Initially, samples were used in the frozen state for DM determination by freeze drying. The resultant dry samples were then used for Kjeldahl nitrogen determination (A.O.A.C., 1965). However, use of the frozen sample directly for nitrogen analysis resulted in more consistent results between triplicates of each sample. Because the total carcass nitrogen value desired did not depend on a DM determination, it was discontinued. To ascertain that differences in carcass nitrogen were not due to nonprotein nitrogen in the body water, urea and ammonia also were determined on each carcass sample, except those from the second trial (31 rats). The samples for this trial were accidentally thawed before these analyses were conducted. Approximately 0.5 g of frozen sample was mixed with 8 ml deionized water to extract the urea and ammonia. The samples were maintained in an ice bath at all times. After centrifugalization at 3000 rpm for 10 minutes at 4°C, the supernatant was decanted and frozen. A modification of the procedures by the Technicon Corporation (1960, 1965) was used for the ammonia and urea determination.

Samples from the aorta and portal vein of each rat were used for the analysis of blood nitrogen components. Ammonia and urea were analyzed by similar procedures as indicated above. Free α amino acids

were determined by a modification of the automated method developed by Palmer and Peters (1966).

No microbial studies were conducted on the cecal contents. Because the type and level of antibiotics were the same as reported by Chawla et al. (1976) it was assumed similar changes occurred. That report indicated no differences in the total count of microbes in the small intestine, but anaerobic microbes were depressed in antibiotic supplemented rats. Kent et al. (1969) with the same antibiotics eliminated E. coli and enterococci from the cecum and bacteroides, entercocci, and lactobacilli from the small intestine.

Due to the varying size and consistency of cecal samples, dilutions with a 0.2M phosphate, 0.001M EDTA buffer (pH 7.0) were made to provide adequate volume for analysis. Original and diluted samples were maintained in ice baths at all times except during the actual weighing and dilution process. The diluted samples were shaken to provide a uniform suspension of debris and an aliquot taken and frozen. Ammonia, urea, and free α amino acids were determined as with the blood samples. These determinations were made using only the liquid portion of the sample. Bacterial and/or feed debris was considered to contribute nothing to these values. An automated procedure was developed for measuring urease activity in these samples by modifying procedures from Szasz (1974), Cook (1976), and Lee (1977). Basically the procedure was the reverse of the urea assay of Szasz (1974). Instead of using urease to determine urea in a sample, urea was added as a substrate to determine urease in the sample. Samples were continuously stirred during sampling in order

that the debris containing any microbial cells and their urease would be a part of the assay. Substrate was supplied by adding 1 mg urea nitrogen/ml solution. Additional ammonia produced above that determined in the unstirred sample was considered to be liberated as the result of urease activity.

Statistical Procedures

Analyses of all data were conducted using the general linear model (GLM) procedures as presented by the SAS Institute (1979). Main effects included in the model were protein level, antibiotic level, trial, and block. All second order interactions, excluding those involving block effect, were also used. Least square procedures were used to estimate means and standard errors in order to compensate for unequal subclass numbers. Since diets containing 7.88% and 21.00% CP were not included in each trial, observations on these diets were deleted from the GLM analysis. Therefore, least square means and standard errors are reported for all other diets. Actual means are reported for the 7.88% and 21.00% diets to provide additional perspective to the data. In order to test differences between protein levels and antibiotic supplementation, the predicted difference option of the GLM procedure was used to conduct a Student's t test between all means of the protein X antibiotic interaction. However, as was planned before the analyses were conducted, the only individual comparisons used were:

1) for an antibiotic effect at each protein level,

2) for a protein effect without antibiotics, and

3) for a protein effect with antibiotics.

Tables presenting the results of different variables in subsequent sections include all significance levels for the comparisons listed above. In addition to the accepted levels of significance, $P<.05$, .01, and .001, the 10% level was considered to indicate a possible difference. Significance levels above 10% were also presented. These levels should only be used as an aid in determining the possibility of similar trends in future research.

Any significant main effect due to either protein level or antibiotic supplementation, independent of the other main effect, will be reported as a general effect due to protein or antibiotic without reference to the other. In some cases, trial and block main effects were significant for the variable in question. However, these effects are not discussed with all variables as they can be attributed to differences in initial weight or analytical analyses. Initial weight varied, as will be discussed in the Results and Discussion section, due to difficulties in starting each trial at the same exact average weight. Samples were stored and analyzed randomly within a trial, and therefore, normal day-to-day variation in analytical procedures could result in differences between trials. In either case, responses were in the same direction across all trials, but not necessarily of the same magnitude.

Preliminary Trials

Results of two preliminary trials need to be mentioned even though no significant contribution to the final data resulted from either. These trials were different from the preliminary trials mentioned earlier in which the intubation procedure was perfected. The trials were designed using diets similar to those in Table 2 except no antibiotics were added. However, nonprotein nitrogen in the form of urea and diammonium citrate were added to provide surplus nitrogen to the lower intestinal microbes. Methionine was also added to cover a suspected increased methionine requirement as described in the Literature Review.

The diets were fed dry in feed cups in each cage. This procedure resulted in the rats scratching extreme amounts of feed out of the cups, especially on the 0.00% protein diets, and quantification of feed intake was impossible. In general, the methionine-nonprotein nitrogen supplementation did not affect gain. Due to the varying intake patterns, it was felt problems existed not only with intake estimation per se, but with reduced nitrogen and energy availability. Therefore, any results would be biased especially at the critical 0.00% protein level, and these trials were discarded. The problems encountered did lead to the procedure previously described for subsequent trials.

RESULTS AND DISCUSSION

Growth Trials

Energy and nitrogen intake

Overall general protein and antibiotic effects on feeding rate and nitrogen intake are presented in Tables 5 and 6, respectively. Both are presented as 14 day means. Feeding rate achieved in the trials is expressed as the maintenance energy requirement (NRC, 1972) times the respective factor indicated. The experiment was designed so energy intake would be approximately 1.8 times the NRC (1972) maintenance energy requirement. This feeding rate is not absolute because the energy content of the diets was estimated to be 411 kcal per 100 g DM. Table 7 shows the individual comparisons for feeding rate. No significant differences due to antibiotics were observed at any protein level; however, the groups fed the lowest level of protein did have a lower energy intake relative to the other protein levels ($P<.001$). The lowered feeding rate at the 0.00% and 21.00% protein levels was due to the greater amounts of diet given by intubation, and the varying DM of the slurry diets used for intubation.

Nitrogen intake is reported on a 14 day basis with the data from the first trial adjusted to 14 days. Total nitrogen intake increased as protein in the diet increased, as would be expected (Table 5). There was also a significant general antibiotic effect ($P<.001$) on nitrogen intake (Table 6). Trial ($P<.001$) and block ($P<.03$) effects

Table 5. Effect of protein level on growth trial variables[a]

Dietary crude protein %	Feeding rate[b,c] Mean[d] g	SE	Significance level[e] P<	Nitrogen intake[b] Mean[d] g	SE	Significance level[e] P<
0.00	1.58	±0.02	-	0.09	±0.03	-
2.62	1.69	±0.01	.001	0.72	±0.02	.001
5.25	1.73	±0.01	.006	1.48	±0.02	.001
10.50	1.72	±0.01	.40	3.05	±0.02	.001
15.75	1.69	±0.01	.10	4.51	±0.02	.001

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Fourteen day basis.

[c]Feeding rate times maintenance energy requirement (NRC, 1972) equals approximate total energy intake (Kcal).

[d]Least square mean ± least square standard error.

[e]Overall protein effect, but compared only to the adjacent lower protein level.

Average daily gain			Final weight		
Mean[d] g	SE	Significance level[e] P<	Mean[d] g	SE	Significance level[e] P<
-1.45	±0.13	-	105.76	±2.38	-
+0.01	±0.08	.001	125.06	±1.49	.001
+1.62	±0.10	.001	147.84	±1.85	.001
+4.56	±0.09	.001	187.55	±1.67	.001
+5.13	±0.12	.001	197.23	±2.17	.001

Table 6. Effect of antibiotic supplementation on growth trial variables[a]

Variable	Antibiotic supplementation	Mean[b]	SE	Significance level[c] P<
Feeding rate[d]	-	1.69[e]	±0.01	.001
	+	1.68[e]	±0.01	
Nitrogen intake, g	-	1.93[e]	±0.01	.001
	+	2.01[e]	±0.01	
Average daily gain, g	-	1.61	±0.06	.001
	+	2.34	±0.07	
Final weight, g	-	147.04	±1.18	.001
	+	158.33	±1.22	

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall antibiotic effect.

[d]Feeding rate times maintenance energy requirement (NRC, 1972) equals approximate total energy intake (Kcal).

[e]Fourteen day basis.

Table 7. Feeding rate and nitrogen intake at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Feeding rate[b] Mean[d] g	SE	Significance level[c] Antibiotic[e] P<	Protein[f] P<
0.00	-	7	1.57	±0.02	.60	-
	+	7	1.59	±0.02		-
2.62	-	11	1.70	±0.01	.20	.001
	+	11	1.68	±0.01		.001
5.25	-	9	1.73	±0.02	.55	.30
	+	11	1.74	±0.01		.002
7.88	-	3	1.70			
	+	3	1.71			
10.50	-	9	1.73	±0.02	.20	.85
	+	10	1.71	±0.01		.10
15.75	-	8	1.71	±0.02	.20	.30
	+	4	1.67	±0.02		.20
21.00	-	3	1.63			
	+	5	1.63			

[a]None supplemented = -, supplemented = +.

[b]Feeding rate times maintenance energy requirement (NRC, 1972) equals approximate total energy intake (Kcal).

[c]Significance level interpretation example using feeding level: Under antibiotic column, the values of 1.57 and 1.59 at 0.00% protein are not significantly different. Under protein column, the value of 1.57 at 0.00% protein without antibiotics was significantly different (P<.001) than the value of 1.70 at 2.62% protein without antibiotics. Also, the value of 1.70 was not significantly different than the value of 1.73 at 5.25% protein without antibiotics and so forth. Similar comparisons were used with antibiotics at each protein level. No significance level was presented for differences between nonadjacent protein levels or between antibiotic supplementation at a different protein level.

[d]Fourteen day least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[e]Antibiotic effect within each protein level.

[f]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Nitrogen intake			
		Significance level[c]	
Mean[d] g	SE	Antibiotic[e] P<	Protein[f] P<
0.07	±0.03	.30	-
0.12	±0.04		-
0.70	±0.02	.25	.001
0.74	±0.02		.001
1.44	±0.03	.08	.001
1.51	±0.02		.001
2.13			
2.29			
2.94	±0.03	.001	.001
3.15	±0.02		.001
4.50	±0.03	.70	.001
4.52	±0.04		.001
5.54			
5.40			

were also significant. Block and trial effects were due to an increase in diet fed as body weight increased. Table 7 shows the individual diet comparisons for nitrogen intake. Nitrogen intake did not differ due to antibiotic supplementation in diets of similar protein level, except the intake with the 10.50% protein diet with antibiotics was higher ($P<.001$) than the intake with the diet without antibiotics. There was a possibility that the difference in nitrogen intake between the 5.25% protein diets was significant ($P<.08$). Nitrogen intake did increase regardless of antibiotic supplementation as protein level in the diet increased. The diets for which no statistics are reported follow the trend established with the other diets.

Growth rate and final weight

Initial body weight of all rats across all trials averaged 125.3 g with no general protein or antibiotic effect evident ($P<.60$). There was a block effect ($P<.04$) on initial body weight since the rats were stratified by weight into each block. Due to differences in adjustment period length between trials, initial body weight varied ($P<.001$) across trials from 119.4 to 130.2 g. This effect did tend to influence other variables directly related to initial weight but did not affect the direction of these responses.

Average daily gain and final body weight relationships to dietary protein level are shown in Figure 7 and Figure 8, respectively. Final weight data for the first trial (10 days) were adjusted to a 14 day basis. The actual data on which Figures 7 and 8 are based, along with

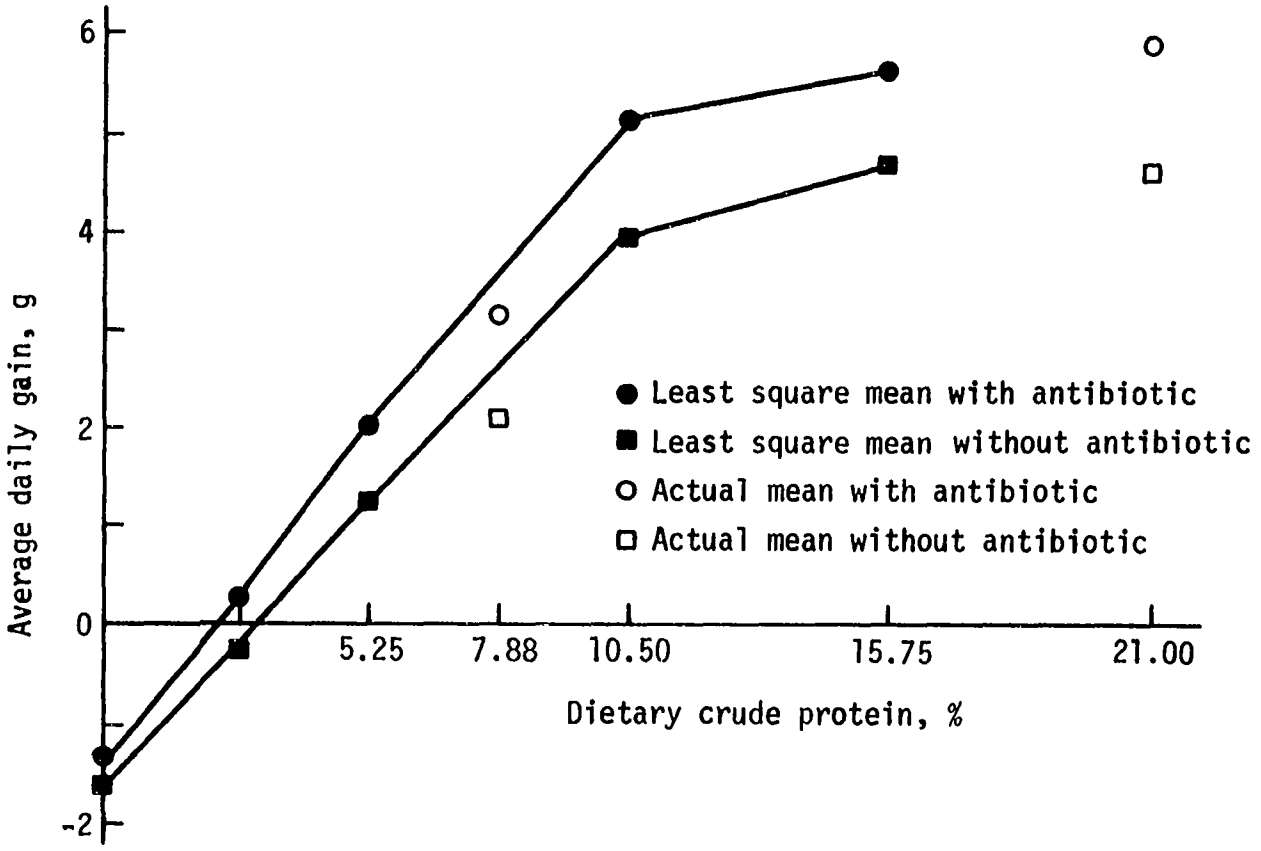

Figure 7. Effect of modified intestinal microflora on average daily gain at different dietary protein levels

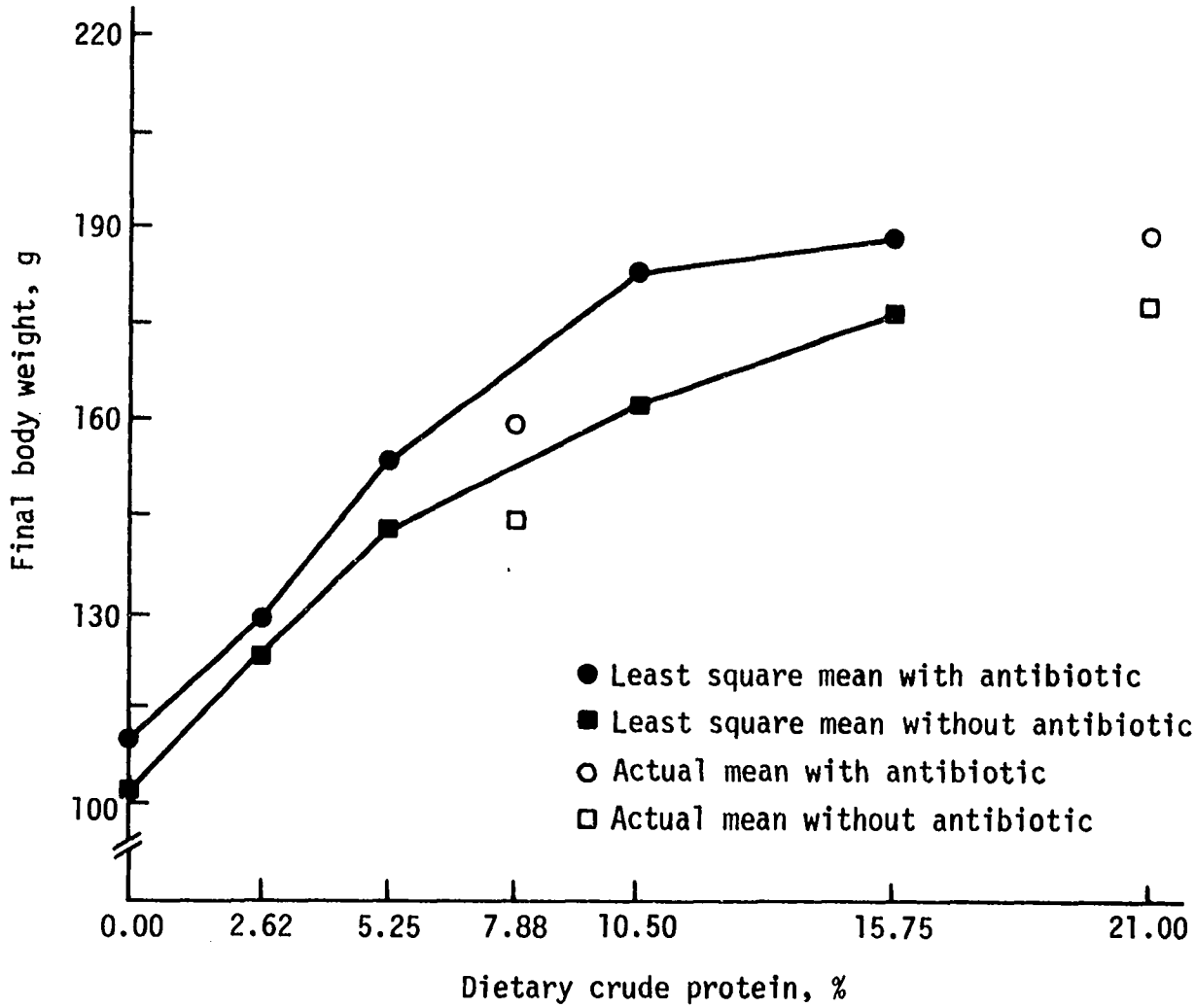

Figure 8. Effect of modified intestinal microflora on final body weight at different dietary protein levels

statistical differences, are shown in Table A1 in the Appendix. Significantly greater daily gains were observed with modified intestinal microflora rats at each protein level with the exception of the lowest protein diet. The increased gain resulted in weights being heavier at the end of the 14 day trial. All final weights were significantly higher for rats having a modified intestinal microflora at any protein level. Also, with increasing protein intake the increase in daily gain and final body weight would be expected (Table 5). Both variables also were increased by antibiotic supplementation ($P<.001$) (Table 6). Because daily gain did not increase at the same rate with both antibiotic supplementations, the protein X antibiotic interaction was significant ($P<.01$). The same interaction was also significant for final body weight ($P<.05$). Therefore, by modifying the intestinal microflora, the rats gained faster and completed the trial at a heavier weight as compared to rats with normal microflora. Research by Pecora (1953), Forbes (1954), Levenson and Tennant (1963), Combe (1973), Riedel (1973), Levenson and Seifter (1974), Chawla *et al.* (1976), Klein *et al.* (1976), Okumura *et al.* (1976), and Veum *et al.* (1978), plus numerous other reports, indicate a positive effect of antibiotic supplementation on growth rate of animals compared to those for nonsupplemented animals. However, at the higher levels of protein, the present study indicated a more pronounced effect of the modification of the microbial population. This is in contrast to reports of a greater advantage at low protein intakes or with poorer quality proteins (Levenson

and Seifter, 1974; Chawla et al., 1976; Klein et al., 1976; Veum et al., 1978).

Carcass Weight and Composition

Carcass weight

In the developmental stages of the present study, nitrogen retention, as determined by carcass nitrogen analysis and by fecal and urine analysis, was included as a part of the experimental procedure. When modification of the intestinal microbes with antibiotics was included as a part of the study, the need to consider the enlarged cecum of rats fed antibiotics was recognized due to a similar problem reported by Chawla et al. (1976). The use of antibiotics also resulted in diarrhea in most antibiotic supplemented rats and precluded the collection of feces and urine. Therefore, the use of carcass weight, body weight minus digestive tract weight, and carcass nitrogen became more important. Three rats, one from each weight stratum, were sacrificed at the beginning of each trial, and this initial slaughter group became the basis for carcass change calculations throughout the study.

Carcass gain (loss) in weight at the different dietary protein levels with and without antibiotic is shown graphically in Figure 9; the actual data and statistical analyses are presented in Table A2 in the Appendix. All weights were reported on a 14 day basis. In contrast to the daily gain and final body weight data, the modification of the microflora in rats fed each protein level had no effect except at the 0.00% protein level. At the 0.00% protein level, rats with

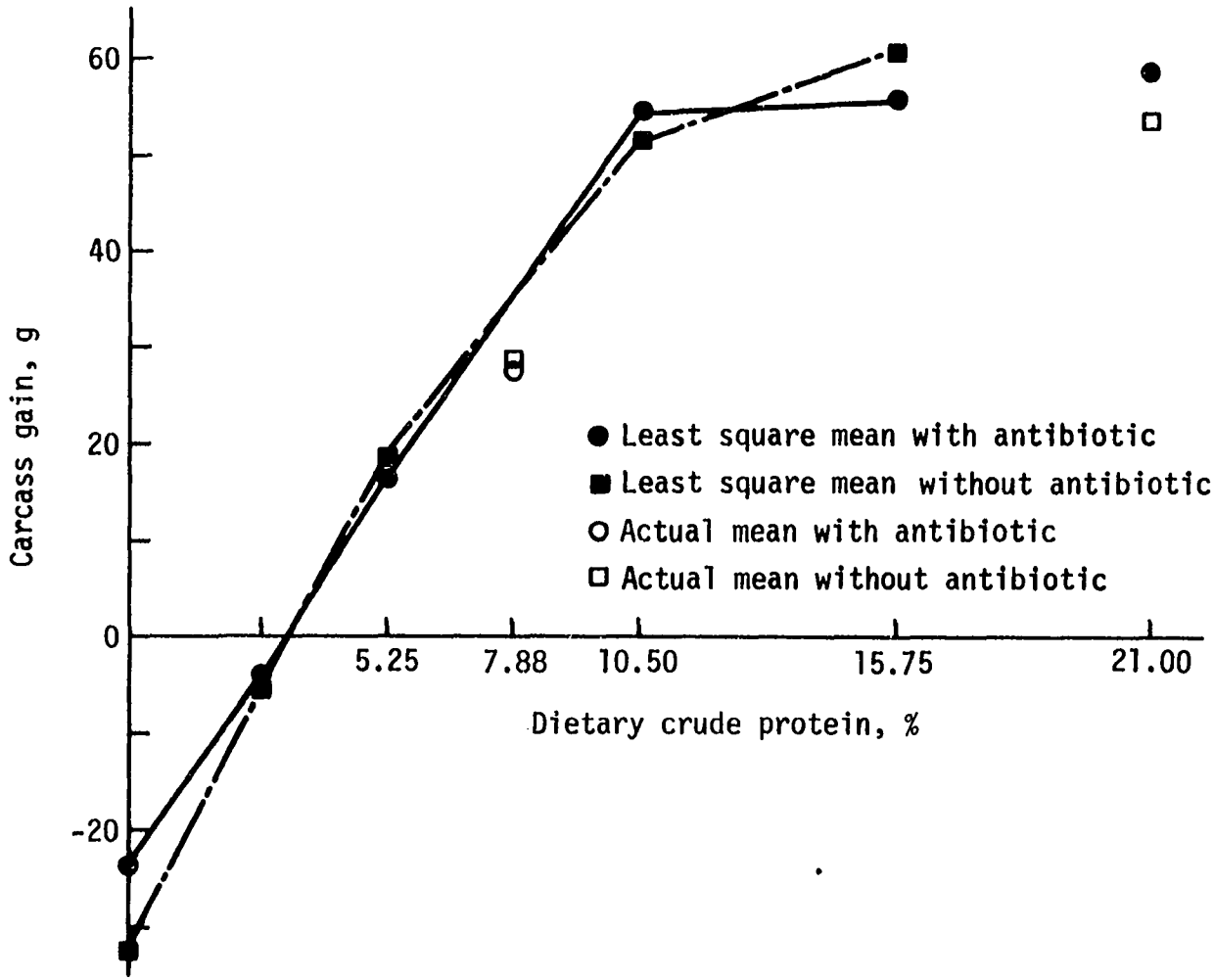

Figure 9. Effect of modified intestinal microflora on carcass gain at different levels of dietary protein

conventional intestinal microbes lost 35% more carcass weight than rats with modified intestinal microflora ($P<.05$). This weight loss was somewhat greater than the 8% increase in gain suggested by Visek (1978) with antibiotic supplemented rats. However, the most important consideration was that the response was only obtained at the lowest (0.00%) protein level. This agrees with the literature reports of antibiotics having the greatest effect at lower protein levels. Therefore, the live weight data presented previously must have been influenced by the intestinal tract weight which will be discussed later. Overall protein level affected carcass gain (loss) (Table 8) while antibiotic supplementation did not ($P<.55$) (Table 9). Actual carcass weight was affected similarly (Tables 8 and 9). Trial effects were significant for carcass weight ($P<.001$) and carcass gain ($P<.002$), indicating the influence of initial weight as mentioned previously. Carcass weight values at 14 days for each dietary protein level are plotted in Figure 10, and the actual data and statistical analyses are presented in Table A2 of the Appendix.

Carcass nitrogen composition

Nitrogen concentration in the carcasses was conducted to estimate any differences in nitrogen retention between treatments. On a wet carcass basis, nitrogen concentration was not significantly different in any of the treatment comparisons (Table A2). However, protein level, regardless of antibiotic supplementation, had a significant influence (Table 8). The concentration of nitrogen in rats fed the 5.25% and

Table 8. Effect of protein level on carcass variables[a]

Dietary crude protein %	Carcass weight Mean[b] g	SE	Significance level[c] P<	Carcass gain (loss) Mean[b] g	SE	Significance level[c] P<
0.00	83.39	±2.42	-	-28.11	±2.37	-
2.62	105.86	±1.51	.001	-4.95	±1.48	.001
5.25	128.23	±1.88	.001	+17.33	±1.85	.001
10.50	163.18	±1.69	.001	+52.69	±1.66	.001
15.75	168.36	±2.21	.07	+58.17	±2.16	.05

Dietary crude protein %	Total carcass nitrogen Mean[b] g	SE	Significance level[c] P<	Total carcass ammonia Mean[b] mmoles	SE	Significance level[c] P<
0.00	2.83	±0.08	-	1.81	±0.45	-
2.62	3.58	±0.05	.001	3.02	±0.25	.03
5.25	4.24	±0.06	.001	3.63	±0.34	.15
10.50	5.45	±0.06	.001	5.99	±0.29	.001
15.75	5.77	±0.07	.001	5.38	±0.37	.20

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

Carcass nitrogen		
Mean[b] %	SE	Significance level[c] P<
3.39	±0.03	-
3.38	±0.02	.80
3.31	±0.03	.04
3.34	±0.02	.40
3.43	±0.03	.04

Total carcass urea		
Mean[b] mmoles	SE	Significance level[c] P<
0.30	±0.06	-
0.31	±0.03	1.00
0.32	±0.05	.90
0.44	±0.04	.04
1.01	±0.05	.001

Table 9. Effect of antibiotic supplementation on variables based on carcass measurements[a]

Variable	Antibiotic supplementation	Mean[b]	SE	Significance level[c] P<
Carcass weight, g	-	129.50	±1.19	.75
	+	130.11	±1.24	
Carcass gain, g	-	18.54	±1.17	.55
	+	19.52	±1.21	
Carcass nitrogen, %	-	3.36	±0.02	.45
	+	3.38	±0.02	
Total carcass nitrogen, g	-	4.35	±0.04	.40
	+	4.40	±0.04	
Total carcass ammonia, mmoles	-	3.86	±0.21	.50
	+	4.07	±0.22	
Total carcass urea, mmoles	-	0.48	±0.03	.95
	+	0.47	±0.03	
Nitrogen retention, g	-	0.86	±0.04	.30
	+	0.92	±0.04	
Thomas-Mitchell biological value, %	-	96.38	±2.92	.001
	+	77.89	±2.80	
New biological value, %	-	82.75	±2.76	.25
	+	77.96	±2.65	
Liver weight, g	-	7.19	±0.16	.55
	+	7.05	±0.16	
Liver weight:carcass weight, %	-	5.75	±0.10	.15
	+	5.53	±0.10	

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall antibiotic effect.

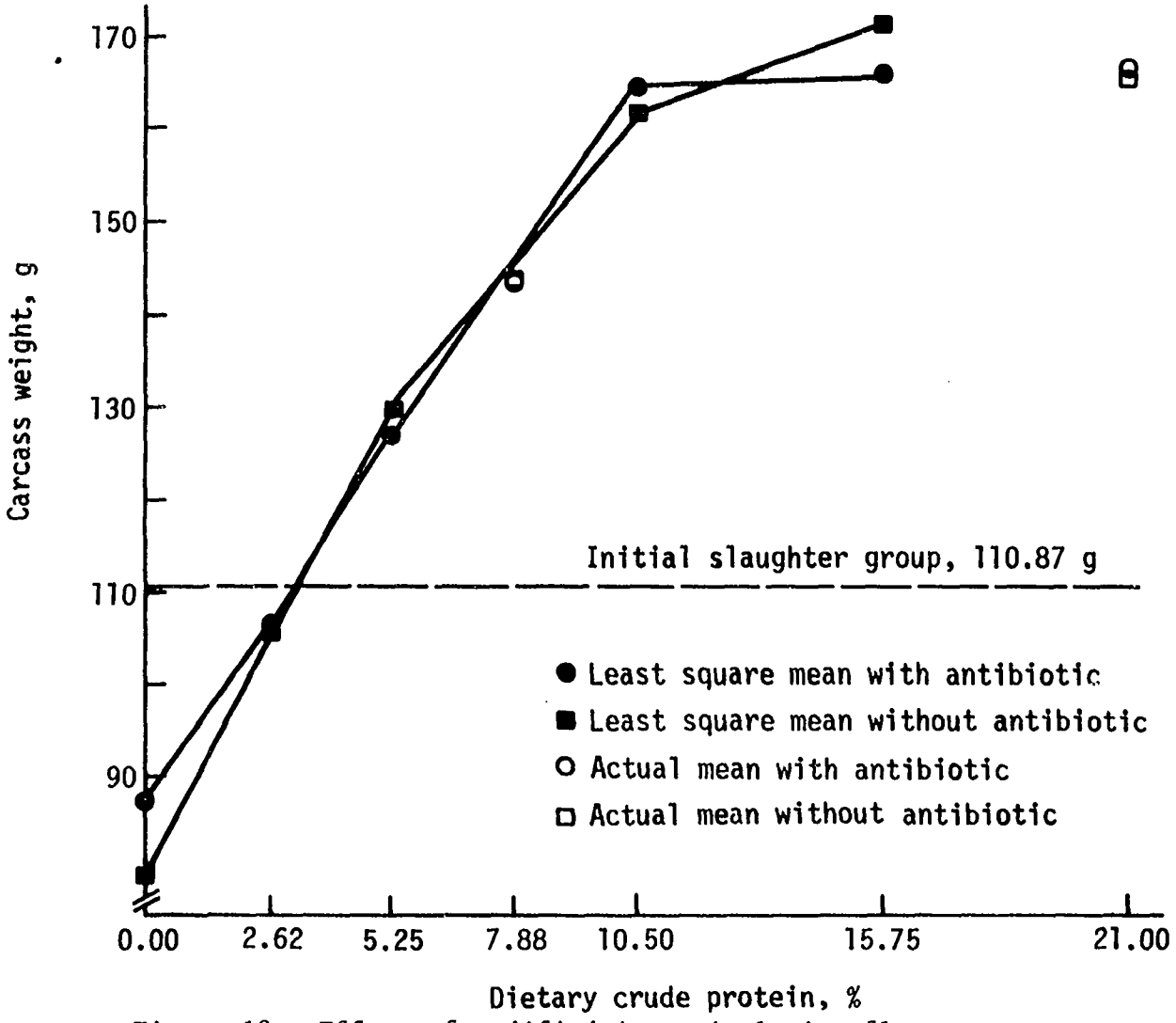

Figure 10. Effect of modified intestinal microflora on carcass weight at different dietary protein levels

10.50% protein diets was lower when compared to the 0.00%, 2.62%, and 15.75% protein diets. Lower carcass urea may be the reason for this response. Multiplying these concentration values times carcass weight resulted in values for total nitrogen per carcass. Since increasing dietary protein over all levels increased carcass weight, a similar response was observed with total carcass nitrogen (Table 8). Antibiotic supplementation had no effect (Table 9) on either total nitrogen ($P<.40$) or nitrogen concentration ($P<.45$). The carcasses from rats fed no protein with a modified microbial population contained 0.3 g additional nitrogen, 2.98 g vs 2.68 g ($P<.02$). These data are shown graphically in Figure 11 with total carcass nitrogen plotted against dietary CP level. The individual comparison data, presented in Table A2, indicated no effect of a modified intestinal microflora at any other protein level. The increases at each protein level for either antibiotic supplementation were significant ($P<.001$), except between the 10.50% and 15.75% protein levels with antibiotics. Figure 12 depicts the relationship of total carcass nitrogen compared to actual nitrogen intake.

Because all the above calculations were based on carcass nitrogen determination by the Kjeldahl method, urea and ammonia determinations were conducted on the carcass samples to ascertain if any differences were due to increases in these compounds, especially urea, because it equilibrates rapidly in the body water. Antibiotic effect at each protein level was not significant for either total carcass urea or ammonia (Table 10), nor was either affected by overall antibiotic supplementation (Table 9). Total carcass urea and ammonia did increase as protein

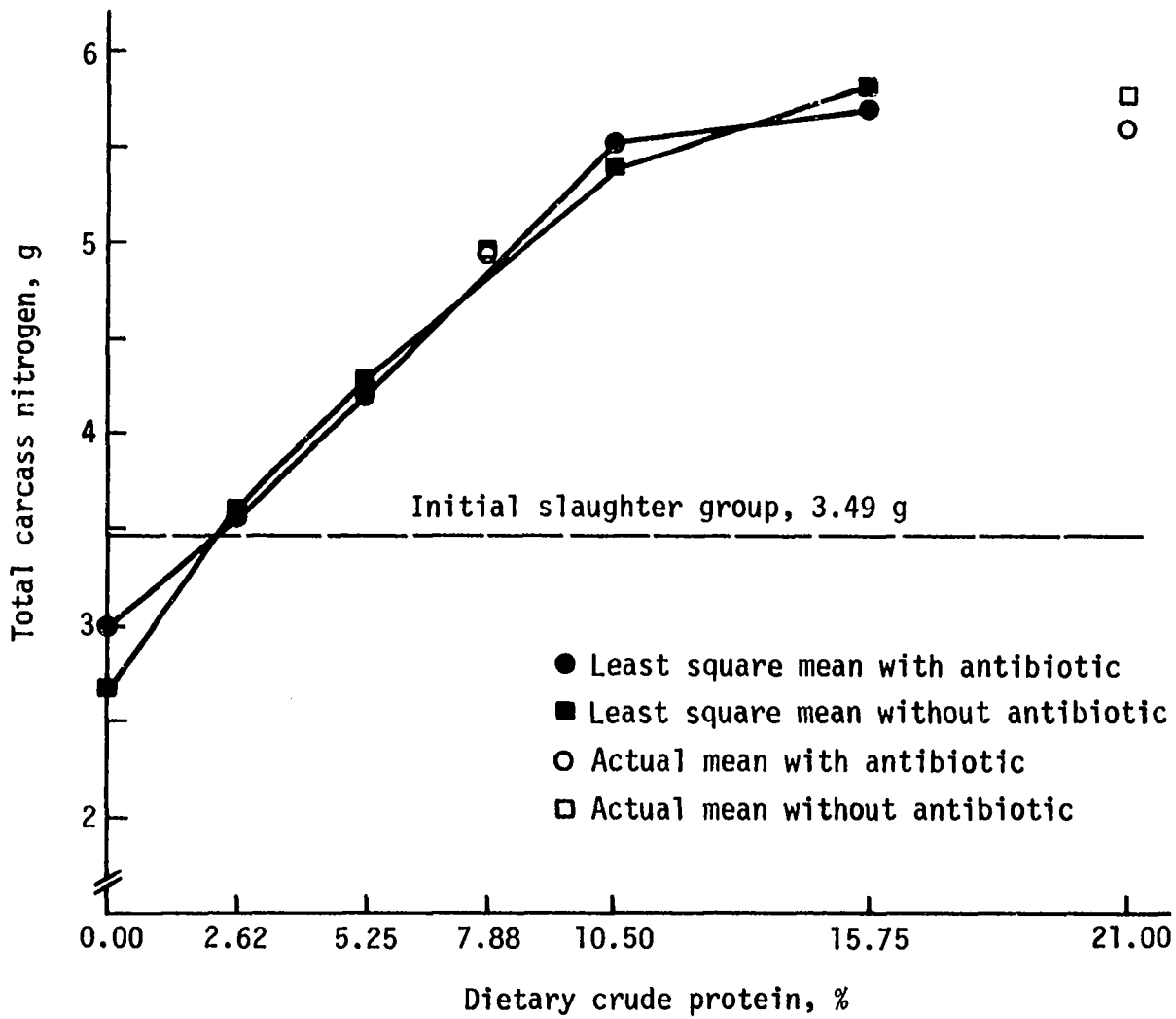

Figure 11. Effect of modified intestinal microflora on carcass nitrogen content at different levels of dietary protein

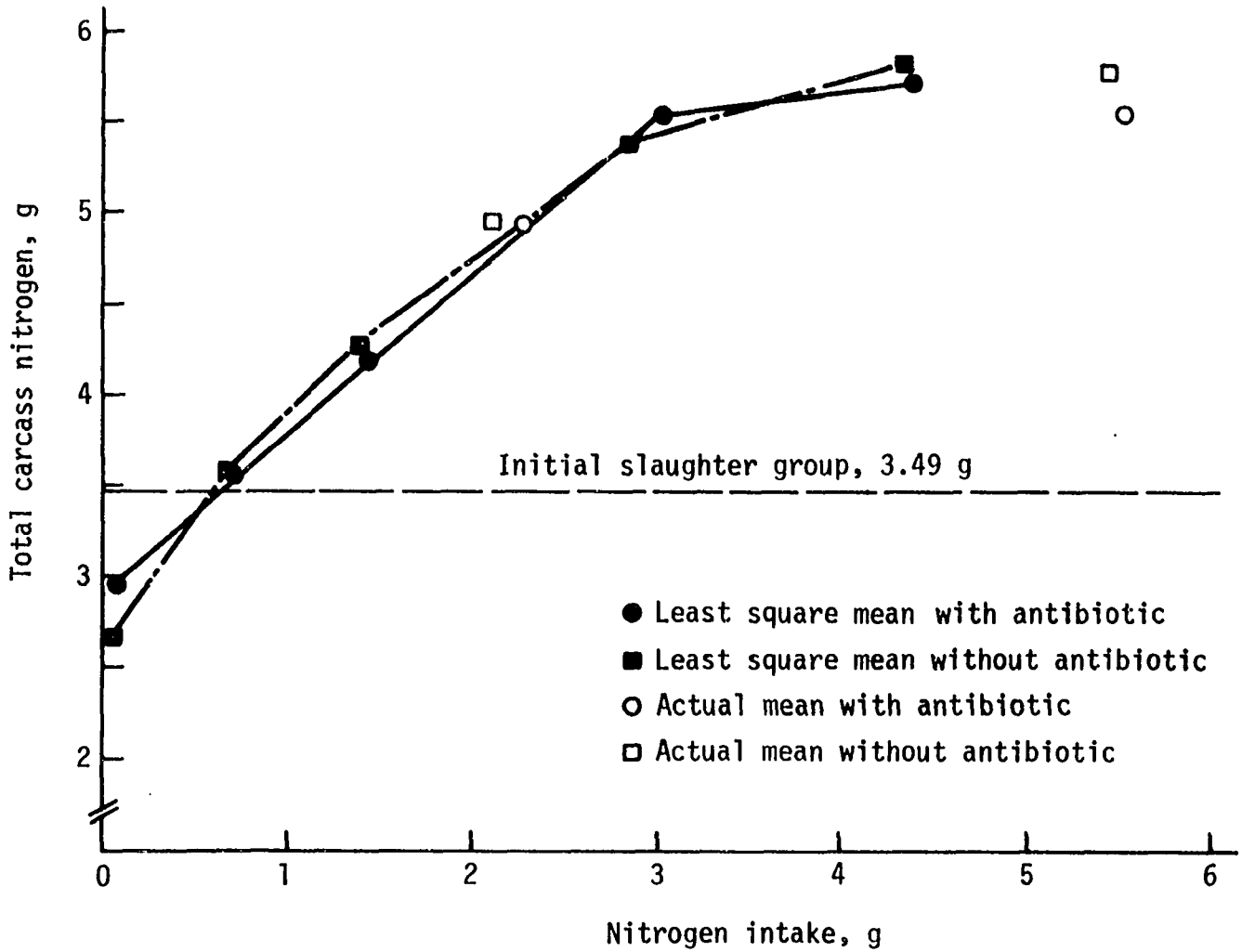

Figure 12. Effect of modified intestinal microflora on total carcass nitrogen at differing levels of nitrogen intake

Table 10. Effect of modified intestinal microflora on total carcass ammonia and urea at different dietary protein levels

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Total carcass ammonia			
			Mean[c] mmoles	SE	Significance level[b] Antibiotic[d] P<	Significance level[b] Protein[e] P<
0.00	-	4	2.15	±0.53	.40	-
	+	4	1.46	±0.67		-
2.62	-	8	2.69	±0.36	.20	.40
	+	8	3.34	±0.36		.02
5.25	-	6	3.79	±0.55	.60	.10
	+	8	3.48	±0.36		.75
7.88	-	3	3.89			
	+	3	4.11			
10.50	-	6	5.79	±0.44	.40	.005
	+	7	6.20	±0.39		.001
15.75	-	6	4.90	±0.44	.20	.15
	+	3	5.87	±0.58		.60
21.00	-	2	5.41			
	+	5	4.61			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Total carcass urea			
		Significance level[b]	
Mean[c] mmoles	SE	Antibiotic[d] P<	Protein[e] P<
0.34	±0.07	.55	-
0.27	±0.09		-
0.31	±0.05	.95	.75
0.30	±0.05		.75
0.34	±0.07	.55	.70
0.29	±0.05		.80
0.27			
0.33			
0.41	±0.06	.35	.50
0.48	±0.05		.01
1.00	±0.05	.75	.001
1.03	±0.07		.001
1.44			
1.44			

level increased with the largest change occurring at the 10.50% protein level for ammonia and the 15.75% level for urea (Table 8). These changes agree with the reduced rate of nitrogen retention per 100 g nitrogen intake at these protein levels (Table 11) and imply the animal was above the protein required for maximal growth at the 10.50% level. However, the increase in total carcass nitrogen at the 0.00% protein level with antibiotics shown in Figure 11 and Table A2 was not due to increases in either urea or ammonia.

From the relationship in Figure 12 and from the calculation of nitrogen retention per 100 g nitrogen intake, Table 11, the response appeared to peak between the 7.88% and 10.50% protein level. Therefore, using the least square means for total carcass nitrogen and nitrogen intake at dietary protein levels of 2.62%, 5.25%, and 10.50%, the following regression equations were calculated:

1) without antibiotics $y = 3.068 + 0.794x$

2) with antibiotics $y = 2.973 + 0.809x$

where y = total carcass nitrogen, g, and

x = nitrogen intake, g.

Using the mean nitrogen intake of 0.118 g with the 0.00% protein diet with antibiotics in equation 2, total carcass nitrogen was predicted to be 3.068 g. The determined value for that diet was 2.984 g, a difference of 0.084 g. Using the mean nitrogen intake of 0.071 g with the 0.00% protein diet without antibiotics in equation 1, total carcass nitrogen was predicted to be 3.124 g. The difference is 0.444 g from the determined value of 2.680 g. Because there was no overall

Table 11. Effect of dietary protein level on nitrogen retention per 100 g nitrogen intake

Dietary crude protein %	Number of rats	N retention / N intake		
		Mean[a] g/100g	SE	Significance level[b] P<
0.00	14	-[c]		
2.62	22	14.05	±3.54	-
5.25	20	48.01	±4.45	.001
7.88	6	63.80		
10.50	19	62.02	±3.97	.02
15.75	12	49.35	±5.17	.06
21.00	8	38.74		

[a]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[b]Overall protein effect, but compared only to the adjacent lower protein level.

[c]No values computed for this protein level.

antibiotic effect on total carcass nitrogen ($P<.40$), the two lines described by the equations above can be assumed to be quite similar. Interestingly, the equation developed from rats with the modified intestinal microflora predicts the total carcass nitrogen of the 0.00% protein group within 2.8% of that actually determined. The difference in corresponding values for the conventional microflora rats was 16.5%. It should be reemphasized that the equations were developed without the benefit of the total carcass nitrogen values at 0.00% protein. The similarity between predicted and actual data is therefore emphasized when the actual data are not used in development of the equation.

These data are similar to those of Harmon et al. (1968) in which the predicted nitrogen retention was 8% and 71% different from the actual value for the germfree and conventional animal, respectively. The regression equations from the data of Harmon et al. (1968) were based on the two highest protein levels due to the possibility of reduced total nitrogen intake with the two lower protein diets as discussed in the Literature Review (Figure 2). Therefore, those data and the data of the present study indicate the nitrogen loss must have been reduced at the lower protein intakes with the modified intestinal microflora. This reduction was to a level that corresponded to a smaller, true maintenance need similar to that occurring at higher protein intakes. The fecal nitrogen excretion data of Harmon et al. (1968) with germfree rats when extrapolated to zero nitrogen intake became almost zero, 0.03 g per g DM intake. By association of these results with those of the present

study, it can be concluded that the MFN excretion in the rats with the modified intestinal microflora was reduced, and probably close to the value determined by Harmon et al. (1968).

Protein evaluation based on carcass composition

Figure 13 presents the relationship of nitrogen retention to dietary protein level. Actual nitrogen retention data and statistical analyses are presented in Table A3. Since nitrogen retention was calculated using carcass nitrogen differences between test animals and their respective initial slaughter animal, similar statistically significant differences were found as with carcass nitrogen. Nitrogen retention was not affected by antibiotic supplementation ($P<.30$), but was increased by increasing overall protein level, Tables 9 and 12, respectively. The only antibiotic effect was a lesser nitrogen loss at the 0.00% protein level ($P<.03$) when the intestinal microflora were modified. Based on the NRC (1972) value for the maintenance nitrogen requirement when feeding an ideal protein, the maintenance nitrogen requirement for the rats used in this study was approximately 1.00 g of nitrogen for 14 days. If it is assumed that the dietary egg protein was perfectly utilized, then this maintenance requirement could be used to predict approximately a 1.00 g loss of nitrogen on a diet devoid of egg protein when fed for 14 days. According to the data in Table A3, the conventional microflora rats lost 0.83 g of nitrogen which could be considered to be similar to the maintenance value reported by NRC (1972). However, modification of the intestinal microflora resulted in only 50%

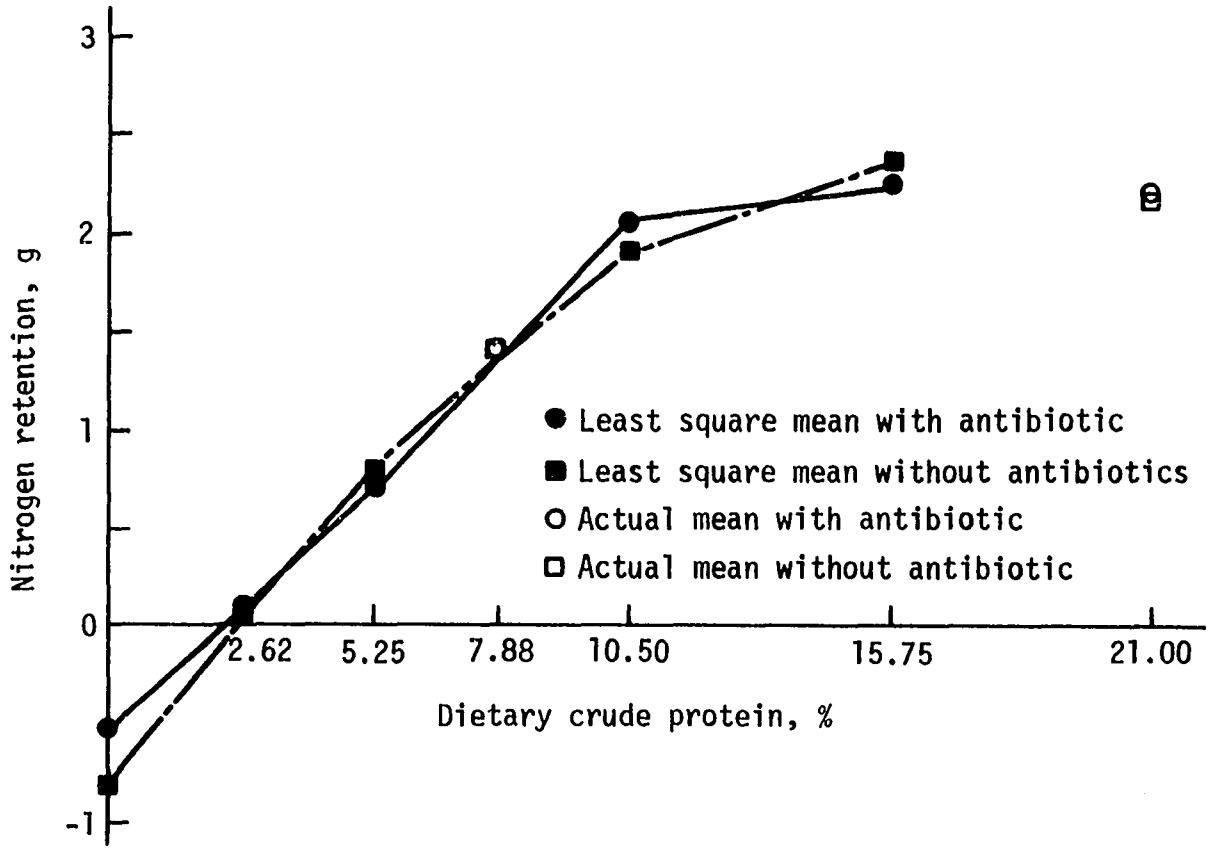

Figure 13. Effect of modified intestinal microflora on 14 day nitrogen retention at differing dietary protein levels

Table 12. Effect of protein level on nitrogen retention, and the Thomas-Mitchell and new biological values[a]

Dietary crude protein %	Nitrogen retention			Thomas-Mitchell biological value		
	Mean[b] g	SE	Significance level[c] P<	Mean[b] %	SE	Significance level[c] P<
0.00	-0.68	±0.08	-	-[d]		-[d]
2.62	+0.09	±0.05	.001	106.90	±3.28	-
5.25	+0.75	±0.06	.001	93.60	±4.15	.02
10.50	+1.98	±0.06	.001	83.66	±3.69	.08
15.75	+2.30	±0.07	.001	64.39	±4.80	.003

Dietary crude protein %	New biological value		
	Mean[b] %	SE	Significance level[c] P<
0.00	-[d]		-
2.62	92.63	±3.11	-
5.25	86.34	±3.92	.25
10.50	80.13	±3.49	.25
15.75	62.32	±4.54	.003

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall antibiotic effect.

[d]No values computed for this protein level.

as much loss as that predicted by NRC (1972). Therefore, this is indirect support for the concept discussed previously in which the maintenance nitrogen, especially the MFN, was influenced by the microbial population of the lower intestine.

Calculation of the biological value of the autoclaved egg protein fed to conventional animals (Harmon et al., 1968) results in values of 61%, 51%, and 44% with diets containing approximately 5%, 10%, and 15% CP from egg albumin. Mitchell (1964) also indicated increased protein intake resulted in declines in biological value. The decline was reported not to be significant until protein intake exceeded that amount needed for maximal growth. However, the decline was found consistently across several trials. Returning to the Harmon et al. (1968) data, a recalculation of the biological values using the MFN value determined with the germfree rats resulted in values of 43%, 42%, and 38% for the 5%, 10%, and 15% protein diets, respectively. Therefore, eliminating the microbial effect on MFN tended to allow the biological value to become somewhat constant up to the protein intake required for maximal growth.

Data from the present study support these results and are graphically shown in Figures 14 and 15 and reported in Table A3 in the Appendix. Figure 14 represents the effect of increasing dietary protein on the Thomas-Mitchell biological value of the diet. That value was determined by the following equation:

$$BV(\%) = \frac{\text{Carcass N test animal} - \text{carcass N protein free animal} + \text{total N intake protein free animal}}{\text{Total N intake test animal}} \times 100.$$

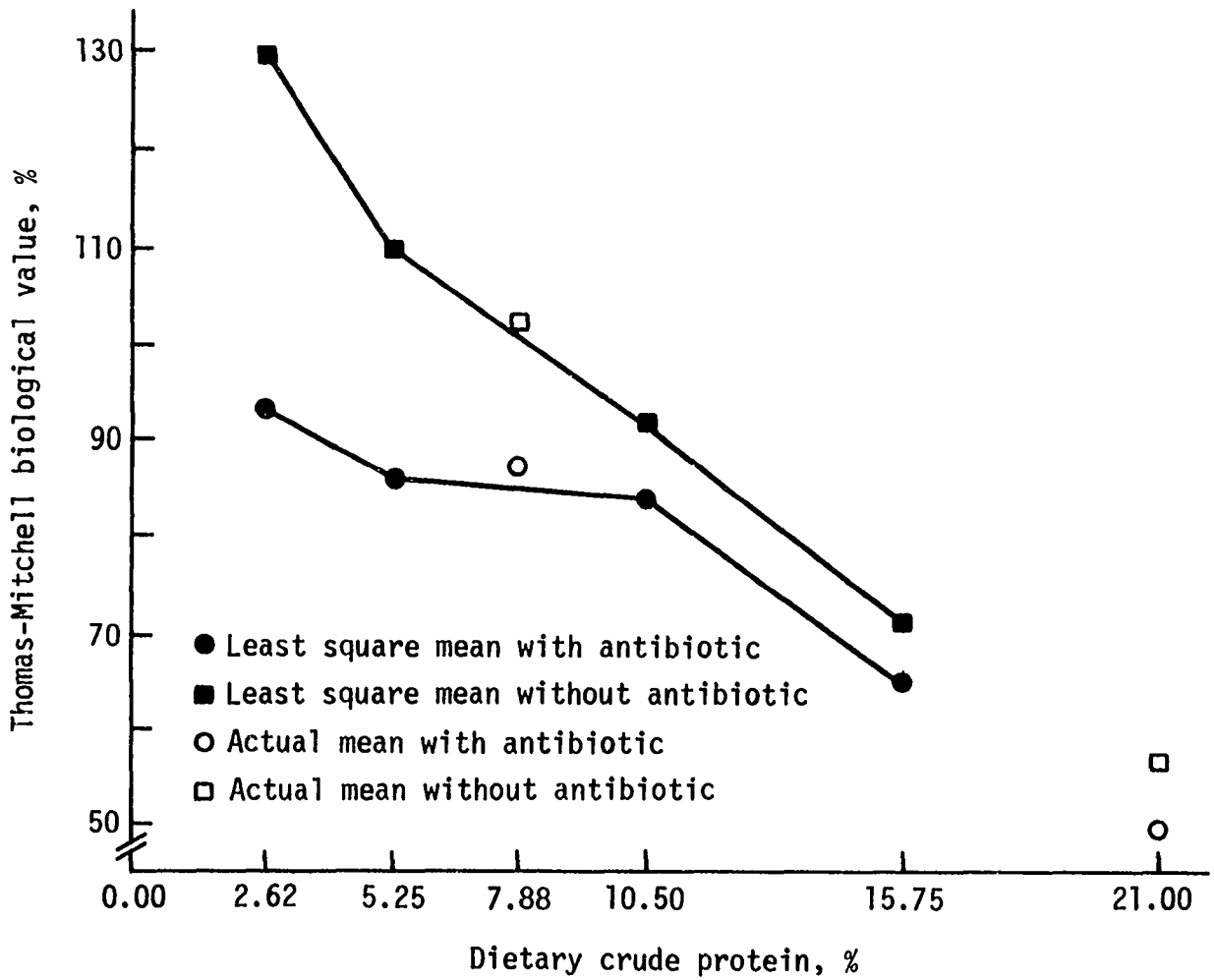

Figure 14. Effect of modified intestinal microflora on the Thomas-Mitchell biological value at differing levels of dietary protein

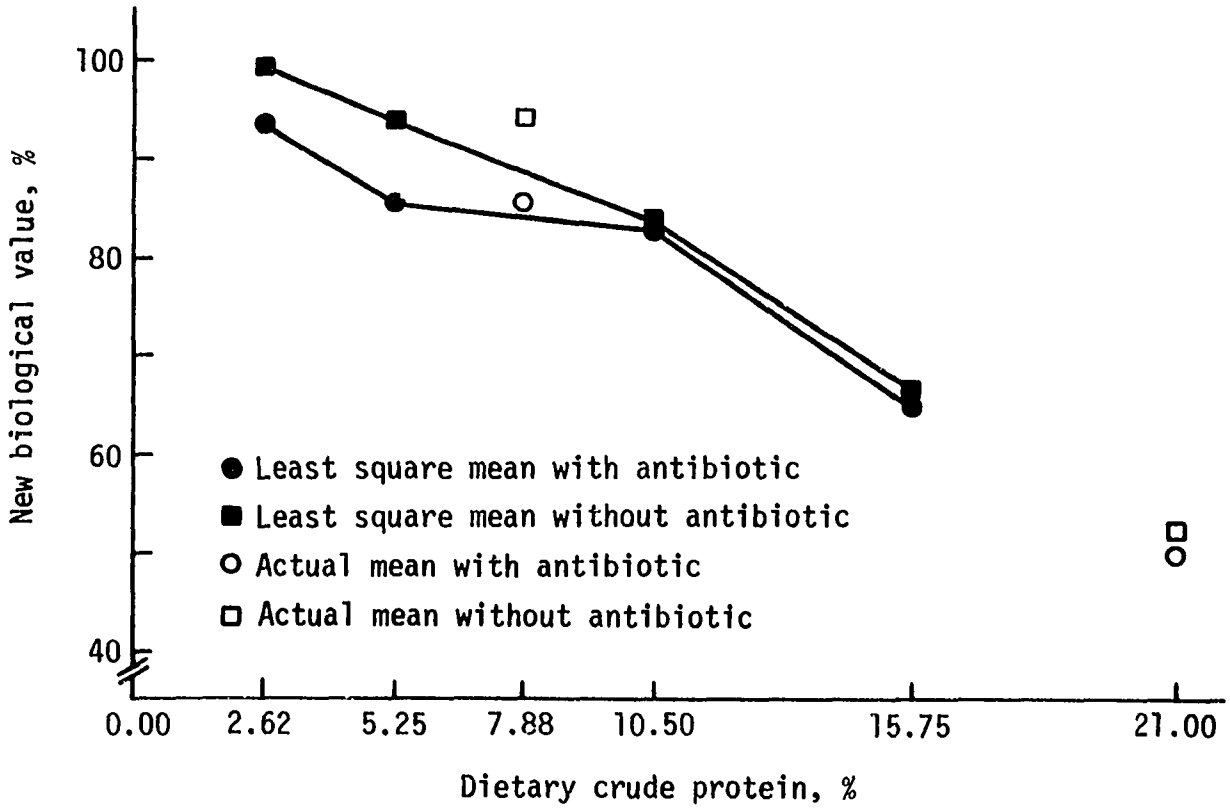

Figure 15. Effect of modified intestinal microflora on a new biological value at different dietary protein levels

The protein free values were from the same level of antibiotic and same block as the test animal. Therefore, credit was given to the egg protein for covering the respective maintenance needs, including MFN. Values of greater than 100% are theoretically impossible, and were due to the method of calculation in this example. In these calculations nitrogen intake and carcass nitrogen for rats in the first trial were not adjusted to a 14 day basis. Biological value was affected by both a general antibiotic and protein effect as indicated in Tables 9 and 12, respectively. Without antibiotics the biological value followed a steady, significant decline across all levels of protein intake (Table A3). However, calculation with the diets containing antibiotics resulted in similar values up to 10.50% dietary protein and then declined in a similar fashion as the values for diets without antibiotics ($P<.55$)

The new biological value relationship depicted in Figure 15, with the actual data in Table A3, was calculated similarly, except the protein free animal with antibiotics was used for the maintenance values at both levels of antibiotics. In this case credit was only given to the maintenance loss in the rats with the modified intestinal microflora. According to the data of Harmon et al. (1968), this should reduce the influence of the MFN losses. General antibiotic effect had no influence on the new biological values ($P<.25$) (Table 9). Above 10.50% protein the values both with and without antibiotics again declined ($P<.05$). This decline was also reflected in the general protein response (Table 12). No difference was observed at any of the lower protein levels.

Therefore, by altering the lower intestinal fermentation, biological value becomes constant below the nitrogen requirement for maximal gain.

These results would suggest the conventional microflora of the intestine "tie up" nitrogen, presumably from intestinal endogenous nitrogen, and cause the nitrogen to be excreted as microbial protein. At higher levels of intake, urea may be recycled into the intestine to cover the microbial need for nitrogen. Therefore, not as much intestinal endogenous nitrogen would be lost in the feces as microbial protein. This would not reduce the total fecal nitrogen loss, but would result in less body α amino nitrogen, or "true" MFN, loss. The remaining intestinal endogenous nitrogen could then be reabsorbed and reutilized.

Liver weight

Due to the liver's central role in the metabolism of absorbed nutrients, liver weights were taken to determine if the expected reabsorption of amino acids with the modified intestinal microflora would influence liver weights. Increasing dietary protein level did increase liver weight (Table 13), but the effect may have been due to the increased carcass weight. Data in Table 9 indicate antibiotics had no effect ($P<.55$).

Individual treatment comparisons, Table 14, resulted in no differences that could be explained as physiologically important. Therefore, the percentage of liver in the carcass was calculated. Liver weights as a percentage of carcass weight decreased as dietary protein

Table 13. Effect of protein level on liver weight and liver weight as a percent of carcass weight[a]

Dietary crude protein %	Liver weight			Liver weight:Carcass weight		
	Mean[b] g	SE	Significance level[c] P<	Mean[b] %	SE	Significance level[c] P<
0.00	5.50	±0.32	-	6.70	±0.20	-
2.62	6.16	±0.20	.09	5.84	±0.12	.001
5.25	6.92	±0.25	.02	5.38	±0.15	.03
10.50	8.19	±0.22	.001	5.02	±0.14	.09
15.75	8.83	±0.29	.09	5.26	±0.18	.35

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

Table 14. Effect of modified intestinal microflora on liver weight and liver weight as a percent of carcass weight at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Liver weight			
			Mean[c] g	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<
0.00	-	7	5.51	±0.38	.95	-
	+	7	5.49	±0.45		-
2.62	-	11	6.11	±0.28	.80	.20
	+	11	6.21	±0.28		.20
5.25	-	9	7.42	±0.38	.03	.007
	+	11	6.43	±0.28		.60
7.88	-	3	7.84			
	+	3	7.39			
10.50	-	9	8.18	±0.32	.95	.15
	+	10	8.20	±0.30		.001
15.75	-	8	8.74	±0.35	.75	.25
	+	4	8.93	±0.46		.20
21.00	-	3	8.37			
	+	5	8.50			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Liver weight:Carcass weight			
		Significance level[b]	
Mean[c] %	SE	Antibiotic[d] P<	Protein[e] P<
6.98	±0.23	.09	-
6.42	±0.27		-
5.82	±0.17	.90	.001
5.85	±0.17		.09
5.72	±0.24	.02	.75
5.04	±0.17		.002
5.47			
5.15			
5.09	±0.20	.60	.04
4.96	±0.18		.75
5.14	±0.22	.50	.85
5.38	±0.29		.20
5.04			
5.11			

increased (Table 13). At the protein level where antibiotic supplementation affected carcass nitrogen, 0.00% protein, the percentage may have been decreased ($P<.09$) (Table 14). As the protein level with or without antibiotics approached that at which maximal carcass gain occurred the percentage was significantly decreased. The decrease occurred with rats having normal microflora at the 10.50% protein level ($P<.04$) and with the modified microflora rats at 5.25% protein ($P<.002$). The decrease at a lower protein level, while no carcass weight difference was evident, may indicate an advantage in the protein metabolism of the modified intestinal microflora animals. The decrease in the percentage of liver in the carcass tended to be lower with all antibiotic treatments ($P<.15$) (Table 9). The lowered liver weight and lower percentage of liver in the carcass with the animals having a modified intestinal flora may be an indication of less amino acid catabolism in the liver. If a higher quality mixture of amino acids was absorbed, less tissue amino acids would need to be catabolized. Admittedly, this conclusion based on these data can be subject to question. A more accurate measure would have been based on the DNA content of the liver or enzyme activities in the liver (Munro and Fleck, 1969).

Cecal Weight and Composition

Cecal weight

Overall protein effects on cecal weights are shown in Table 15. As expected, the ceca of antibiotic supplemented rats were quite large. By visual observation, the increase in size seemed to be limited to the

Table 15. Effect of protein level on cecal weights[a]

Dietary crude protein %	Total cecal weight Mean[b] g	SE	Significance level[c] P<	Cecal content Mean[b] g	SE	Significance level[c] P<
0.00	4.93	±0.63	-	4.09	±0.58	-
2.62	5.91	±0.39	.20	5.03	±0.36	.20
5.25	6.16	±0.47	.70	5.15	±0.44	.85
10.50	7.69	±0.42	.02	6.34	±0.40	.05
15.75	8.35	±0.55	.35	6.97	±0.52	.35

Dietary crude protein %	Cecal tissue Mean[b] g	SE	Significance level[c] P<
0.00	0.83	±0.07	-
2.62	0.87	±0.04	.60
5.25	1.02	±0.05	.03
10.50	1.35	±0.05	.001
15.75	1.38	±0.06	.70

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

cecum with very little distension in the small intestine. Some enlargement could be detected in the colon. The magnitude of the increase can be seen in Table 16 as antibiotics increased total cecal weight by five-fold ($P<.001$). The consistency of the cecal fluid was watery in rats supplemented with antibiotics, while somewhat thick and viscous in rats not supplemented with antibiotics. These results agree with those of van der Waaij (1969), Coates (1973), Schaedler (1973), Wostmann et al. (1973), Chawla et al. (1976), Visek (1978), and Refat (1978). Therefore, the use of carcass weight and carcass gain (Table A2) was the most reliable estimate of the true effect of the modified intestinal microflora on body weight changes.

Increasing protein level had no effect on total cecal weight (Table 17) in rats with a normal microflora. However, modifying the intestinal microflora allowed the total cecal weight to increase significantly at each protein level up to approximately 7.88% to 10.50% protein in the diet.

Weight of the cecal contents responded similarly to protein level with and without antibiotics and to antibiotic supplementation within protein levels as did total cecal weight (Table 17). There was a trend for the content weight to decrease when no antibiotics were fed up to 10.50% protein. However, none of the weights was significantly different from the adjacent lower protein level. It is possible that this trend was due to a slower rate of passage through the digestive tract with lessened intake in the lighter rats. It would have been desirable

Table 16. Effect of antibiotic supplementation on cecal weight variables[a]

Variable	Antibiotic supplementation	Mean[b]	SE	Significance level[c] P<
Total cecal weight, g	-	2.20	±0.30	.001
	+	11.01	±0.32	
Cecal content weight, g	-	1.47	±0.28	.001
	+	9.56	±0.30	
Cecal tissue weight, g	-	0.74	±0.03	.001
	+	1.44	±0.03	
Cecal content:final weight, %	-	1.04	±0.19	.001
	+	6.04	±0.21	
Cecal content:carcass weight, %	-	1.22	±0.25	.001
	+	7.40	±0.27	
Cecal content:liver weight, %	-	20.18	±5.25	.001
	+	139.61	±5.57	
Cecal tissue:final weight, %	-	0.51	±0.02	.001
	+	0.91	±0.02	
Cecal content:carcass weight, %	-	0.60	±0.03	.001
	+	1.10	±0.03	
Cecal content:liver weight, %	-	10.57	±0.60	.001
	+	21.30	±0.62	

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall antibiotic effect.

Table 17. Effect of modified intestinal microflora on cecal weights at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Total cecal weight			
			Mean[c] g	SE	Significance level[b] Antibiotic[d] P<	Significance level[b] Protein[e] P<
0.00	-	7	3.03	±0.72	.001	-
	+	7[f]	6.83	±0.92		-
2.62	-	11	2.07	±0.54	.001	.30
	+	11[f]	9.74	±0.56		.009
5.25	-	9	1.22	±0.74	.001	.35
	+	11	11.10	±0.54		.09
7.88	-	3	1.63			
	+	3	13.56			
10.50	-	9	1.83	±0.62	.001	.50
	+	10	13.54	±0.56		.003
15.75	-	8	2.85	±0.66	.001	.25
	+	4	13.84	±0.88		.80
21.00	-	3	3.81			
	+	5	11.80			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

[f]One less rat used in total cecal and cecal content analyses.

Cecal content				Cecal tissue			
		Significance level[b]				Significance level[b]	
Mean[c] g	SE	Antibiotic[d] P<	Protein[e] P<	Mean[c] g	SE	Antibiotic[d] P<	Protein[e] P<
2.30	±0.67	.001	-	0.74	±0.08	.15	-
5.89	±0.86		-	0.91	±0.09		-
1.47	±0.50	.001	.35	0.60	±0.06	.001	.20
8.60	±0.53		.009	1.13	±0.06		.05
0.60	±0.69	.001	.30	0.63	±0.08	.001	.75
9.70	±0.50		.15	1.40	±0.06		.002
1.02				0.61			
12.01				1.55			
1.06	±0.58	.001	.60	0.78	±0.07	.001	.15
11.62	±0.53		.02	1.92	±0.06		.001
1.93	±0.62	.001	.30	0.92	±0.07	.001	.15
12.00	±0.82		.70	1.84	±0.10		.50
2.92				0.90			
10.14				1.66			

to express the content weight on a DM basis; however, the small total quantity of cecal contents from rats with a normal microflora reduced the amount of sample that could be saved. The nitrogen analyses, to be discussed below, were felt to be of foremost importance, and DM determinations had to be sacrificed. Dilution of the sample was required for the nitrogen analyses, and an attempt to determine DM on the diluted sample resulted in large variations between triplicates. Use of chromic oxide as a marker, to which other analyses could be compared, was also unsuccessful due to small sample sizes. Therefore, the discussion of the cecal data is limited to a wet basis or total amount per cecum (rat). Wostmann et al. (1973) indicated that the supplementation of antibiotics in rats decreased cecal content DM from approximately 25% to 10%. Combe and Gordon (1969) reported the DM content of the germfree cecum to be 18%. The osmolality was similar to the blood plasma and may have indicated an initial movement of water into the cecum, expanding the cecal wall, but equalizing the osmolality in the process. Miniats and Valli (1973) determined the DM of the colon contents of germfree pigs, SPF pigs, and conventional pigs to be 10.3%, 13.4%, and 21.0%, respectively. Visek (1978) reported about 90% as much DM in the small intestine of germfree animals as compared to conventional animals. Therefore, the cecal contents in the present study may have contained less DM when antibiotics were supplemented. Based on the literature cited above, the decrease in DM was probably no greater than a 50% reduction.

Table 17 also contains data that indicated an increase in cecal tissue weight with the modified intestinal microflora at each protein level ($P<.001$), with the possible exception of the 0.00% protein level. With normal intestinal microflora protein level had no effect on cecal tissue weight, but cecal tissue weight was increased with increasing protein level when the intestinal microflora was modified. Francois (1959) reviewed several articles in which antibiotics caused a thinning of the intestinal mucosa. It was then suggested the thinning allowed more amino acid absorption. However, the increase in cecal tissue weight when antibiotics were fed in the present experiment does not confirm this mucosal thinning theory, yet the antibiotic supplemented animals still deposited more total carcass nitrogen.

In an attempt to remove any effect of increased body weight, cecal content and tissue weight were expressed as a percentage of both final and carcass weights. The percentages calculated and statistical analyses indicated that any effect of protein and/or antibiotic is primarily due to the original cecal and tissue weight. Cecal content as a percentage of final or carcass weight with the modified intestinal microflora was 6.04% and 7.40%, respectively (Table 16). With the normal microflora, the values were 1.04% and 1.22% for the percentage of cecal contents in the final and carcass weights, respectively. The results were similar to the total cecal percentage of 1.0% reported by Loesche (1968) for normal rats, but were lower than the 18% report for antibiotic supplemented rats. Combe and Gordon (1969) reported that the cecum plus

contents in germfree rats accounted for 11% of the final weight. Reports by Savage and Dubos (1968), Loeschke et al. (1973), and Chawla et al. (1976) put the percentage at less than 5% of the body weight. Overall protein effects are presented in Table 18.

Individual treatment comparisons indicated increases due to antibiotics at each protein level for both cecal contents (Table 19) and cecal tissue (Table 20) as a percentage of both final and carcass weight. The physiological significance of these differences is unknown.

Expressing either cecal contents or cecal tissue as a percentage of the liver weight (Tables 16, 18, 19, and 20) resulted in similar trends as discussed above. The values are probably more related to the cecal weight because the liver weight between treatments was not greatly different (Table 14). No physiological basis can be ascribed to the differences reported.

Cecal nitrogen composition

Cecal nitrogen concentrations were calculated on a per gram of wet sample basis, and the concentration multiplied by the cecal content weight to give the total quantity per cecum (rat). Dietary protein increased ammonia concentration (Table 21). Cecal ammonia concentration was not affected by antibiotic supplementation ($P<.20$) (Table 22). However, antibiotic supplementation at individual protein levels did increase cecal ammonia at the 10.50% ($P<.05$) and possibly the 15.75% ($P<.08$) dietary protein levels (Table 23). This was probably the result of a greater ammonia production in the body proper, and a

Table 18. Effect of protein level on cecal content and tissue weights as a percent of final, carcass, and liver weights[a]

Dietary crude protein %	Cecal content:Final weight Mean[b] %	SE	Significance level[c] P<	Cecal content:Carcass weight Mean[b] %	SE	Significance level[c] P<
0.00	3.73	±0.40	-	4.77	±0.52	-
2.62	3.96	±0.25	.65	4.82	±0.33	.95
5.25	3.32	±0.31	.15	3.92	±0.40	.09
10.50	3.24	±0.27	.90	3.86	±0.35	.95
15.75	3.47	±0.36	.65	4.19	±0.46	.60

Dietary crude protein %	Cecal tissue:Final weight Mean[b] %	SE	Significance level[c] P<	Cecal tissue:Carcass weight Mean[b] %	SE	Significance level[c] P<
0.00	0.79	±0.04	-	0.99	±0.05	-
2.62	0.69	±0.03	.06	0.81	±0.03	.006
5.25	0.68	±0.03	.85	0.79	±0.04	.70
10.50	0.70	±0.03	.60	0.82	±0.04	.60
15.75	0.70	±0.04	.90	0.83	±0.05	.95

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

Cecal content:Liver weight		
Mean[b] %	SE	Significance level[c] P<
71.83	±10.93	-
85.16	± 6.82	.35
79.80	± 8.29	.65
81.98	± 7.42	.85
80.71	± 9.66	.95

Cecal tissue:Liver weight		
Mean %	SE	Significance level P
15.52	± 1.22	-
14.85	± 0.76	.65
15.70	± 0.95	.50
17.29	± 0.85	.25
16.30	± 1.11	.50

Table 19. Effect of modified intestinal microflora on cecal content weight as a percent of final, carcass, and liver weights at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Cecal content:Final weight			
			Mean[c] %	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<
0.00	-	7	2.14	±0.47	.001	-
	+	6	5.32	±0.59		-
2.62	-	11	1.22	±0.35	.001	.15
	+	10	6.70	±0.36		.05
5.25	-	9	0.25	±0.48	.001	.15
	+	11	6.38	±0.34		.55
7.88	-	3	0.64			
	+	3	6.91			
10.50	-	9	0.60	±0.40	.001	.55
	+	10	5.88	±0.36		.30
15.75	-	8	1.00	±0.43	.001	.50
	+	4	5.94	±0.57		.90
21.00	-	3	1.54			
	+	5	5.02			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Cecal content:Carcass weight				Cecal content:Liver weight			
		Significance level[b]				Significance level[b]	
Mean[c] %	SE	Antibiotic[d] P<	Protein[e] P<	Mean[c] %	SE	Antibiotic[d] P<	Protein[e] P<
2.80	±0.60	.001	-	42.64	±12.64	.003	-
6.74	±0.77		-	101.02	±16.08		-
1.43	±0.45	.001	.08	24.26	± 9.39	.001	.25
8.21	±0.47		.15	146.06	± 9.16		.02
0.16	±0.62	.001	.10	-0.87	±12.90	.001	.15
7.69	±0.45		.40	160.47	± 9.43		.30
0.71				13.07			
8.41				165.16			
0.62	±0.52	.001	.55	13.08	±10.83	.001	.40
7.09	±0.47		.35	150.88	± 9.87		.50
1.09	±0.55	.001	.55	21.80	±11.58	.001	.60
7.29	±0.74		.80	139.63	±15.41		.55
1.77				36.41			
6.24				122.67			

Table 20. Effect of modified intestinal microflora on cecal tissue as a percent of final, carcass, and liver weights at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Cecal tissue:Final weight Mean[c] %	SE	Significance level[b] Antibiotic[d] P<	Significance level[b] Protein[e] P<
0.00	-	7	0.72	±0.05 5	.07	-
	+	7	0.85	±0.06		-
2.62	-	11	0.49	±0.04	.001	.001
	+	11	0.88	±0.04		.75
5.25	-	9	0.43	±0.05	.001	.40
	+	11	0.92	±0.04		.45
7.88	-	3	0.38			
	+	3	0.89			
10.50	-	9	0.44	±0.04	.001	.90
	+	10	0.96	±0.04		.45
15.75	-	8	0.48	±0.05	.001	.60
	+	4	0.91	±0.06		.50
21.00	-	3	0.48			
	+	5	0.82			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Cecal tissue:Carcass weight				Cecal tissue:Liver weight			
		Significance level[b]				Significance level[b]	
Mean[c] %	SE	Antibiotic[d] P<	Protein[e] P<	Mean[c] %	SE	Antibiotic[d] P<	Protein[e] P<
0.93	±0.06	.20	-	13.83	±1.45	.15	-
1.04	±0.07		-	17.21	±1.72		-
0.56	±0.05	.001	.001	10.24	±1.08	.001	.06
1.06	±0.05		.85	19.46	±1.08		.30
0.48	±0.06	.001	.30	8.09	±1.47	.001	.25
1.11	±0.05		.50	23.30	±1.08		.02
0.43				7.83			
1.09				21.40			
0.49	±0.05	.001	.95	9.98	±1.24	.001	.35
1.16	±0.05		.45	24.60	±1.13		.45
0.54	±0.06	.001	.55	10.68	±1.33	.001	.70
1.12	±0.07		.70	21.91	±1.77		.25
0.55				11.60			
1.01				20.92			

Table 21. Effect of protein level on cecal nitrogen levels[a]

Dietary crude protein %	Cecal ammonia Mean[b] µmoles/g[d]	SE	Significance level[c] P<	Cecal urea Mean[b] µmoles/g[d]	SE	Significance level[c] P<
0.00	8.55	±2.57	-	3.85	±0.88	-
2.62	15.91	±1.45	.02	4.96	±0.50	.30
5.25	21.02	±1.40	.02	6.26	±0.48	.07
10.50	32.38	±1.52	.001	5.79	±0.52	.55
15.75	36.21	±2.19	.20	6.00	±0.75	.85

Dietary crude protein %	Total cecal ammonia Mean[b] µmoles	SE	Significance level[c] P<	Total cecal urea Mean[b] µmoles	SE	Significance level[c] P<
0.00	35.72	±19.01	-	17.64	±7.64	-
2.62	75.26	±10.74	.08	27.87	±4.32	.30
5.25	122.86	±10.35	.003	41.82	±4.16	.03
10.50	239.25	±11.26	.001	46.79	±4.52	.45
15.75	289.56	±16.21	.02	54.55	±6.51	.35

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

[d]Per g cecal contents.

Cecal free amino acids		
Mean[b] µmoles/g[d]	SE	Significance level[c] P<
26.41	±5.95	-
52.32	±3.36	.001
69.52	±3.24	.001
70.29	±3.52	.90
76.78	±5.07	.30

Total cecal free amino acid			Total cecal soluble nitrogen		
Mean[b] µmoles	SE	Significance level[c] P<	Mean[b] mg	SE	Significance level[c] P<
131.37	±71.06	-	4.67	±2.35	-
315.08	±40.16	.03	10.65	±1.32	.04
507.05	±38.69	.001	17.07	±1.28	.002
681.64	±42.08	.004	23.73	±1.39	.001
770.77	±60.59	.25	27.14	±2.00	.20

Table 22. Effect of antibiotic supplementation on cecal nitrogen variables[a]

Variable	Antibiotic supplementation	Mean[b]	SE	Significance level[c] P<
Cecal ammonia, μmoles/g[d]	-	21.72	± 1.06	.20
	+	23.91	± 1.23	
Cecal urea, μmoles/g[d]	-	4.01	± 0.36	.001
	+	6.74	± 0.42	
Cecal free amino acids, μmoles/g[d]	-	34.20	± 2.45	.001
	+	83.93	± 2.84	
Total cecal ammonia, μmoles	-	34.48	± 7.83	.001
	+	270.58	± 9.09	
Total cecal urea, μmoles	-	5.50	± 3.15	.001
	+	69.97	± 3.65	
Total cecal free amino acids, μmoles	-	53.23	±29.27	.001
	+	909.13	±33.97	
Total soluble cecal nitrogen, mg	-	2.13	± 0.97	.001
	+	31.18	± 1.12	

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

[d]Per g cecal contents.

Table 23. Effect of modified intestinal microflora on cecal ammonia, urea, and free amino acid concentration at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Cecal ammonia			
			Mean[c] µmoles/g[f]	SE	Significance level[b] Antibiotic[d] P<	Significance level[b] Protein[e] P<
0.00	-	5	10.72	±2.97	.35	-
	+	5	6.39	±3.64		-
2.62	-	9	16.12	±1.98	.90	.15
	+	8	15.70	±2.12		.04
5.25	-	9	20.26	±1.98	.60	.15
	+	9	21.79	±1.98		.05
7.88	-	3	15.06			
	+	3	38.06			
10.50	-	8	29.30	±2.13	.05	.003
	+	8	35.47	±2.13		.001
15.75	-	6	32.23	±2.61	.08	.40
	+	3	40.19	±3.48		.30
21.00	-	2	27.22			
	+	2	33.13			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

[f]Per g cecal contents.

Cecal urea				Cecal free amino acids			
		Significance level[b]				Significance level[b]	
Mean[c] µmoles/g[f]	SE	Antibiotic[d] P<	Protein[e] P<	Mean[c] µmoles/g[f]	SE	Antibiotic[d] P<	Protein[e] P<
2.88	±1.02	.20	-	19.54	±6.89	.20	-
4.82	±1.25		-	33.28	±8.44		-
4.05	±0.68	.08	.35	35.59	±4.58	.001	.06
5.86	±0.73		.50	69.06	±4.92		.001
4.96	±0.68	.009	.40	44.62	±4.58	.001	.20
7.57	±0.68		.10	94.42	±4.58		.001
4.09				34.89			
6.91				119.84			
4.49	±0.73	.02	.65	34.21	±4.93	.001	.15
7.09	±0.73		.65	106.37	±4.93		.09
3.67	±0.90	.003	.50	37.02	±6.00	.001	.75
8.33	±1.19		.40	116.53	±8.06		.30
3.91				30.98			
7.55				84.17			

reduced microbial utilization of the ammonia in the cecum. No effect at the lower levels was probably due to less total ammonia diffusing into the cecum. This conclusion is somewhat substantiated by the blood nitrogen values presented later. Increases in cecal ammonia with increasing protein were observed at all but the higher protein level (Table 23) when the intestinal microflora were modified and were probably a reflection of the reduced fermentation. On a total cecal basis increasing protein and antibiotics increased the total amount of cecal ammonia (Tables 21 and 22). Individual treatment comparisons indicated no significant protein effect without antibiotics as depicted in Figure 16; actual data and statistical analyses shown in Table A4 of the Appendix. However, at each higher level of dietary protein, modifying the intestinal microflora resulted in a greater increase in cecal ammonia quantity. Increased cecal content weight (Table 16) contributed a large portion to the increased total level of ammonia as both were increased about sevenfold with antibiotic supplementation. The lower value at 21.00% protein was possibly due to only two rats, both from the same trial, contributing to this value. These ammonia concentrations are somewhat different from those of Henderickx and Decuypere (1973), Vervaeke et al. (1976), and Combe as reported by Refat (1978). Those reports indicated the fresh cecal contents contained a 10 times higher concentration of ammonia in the germfree animal than in the conventional animal. The difference between the literature reports and the present study could be due to the modified microflora still utilizing some ammonia, while none was used in the intestine of the germfree animal.

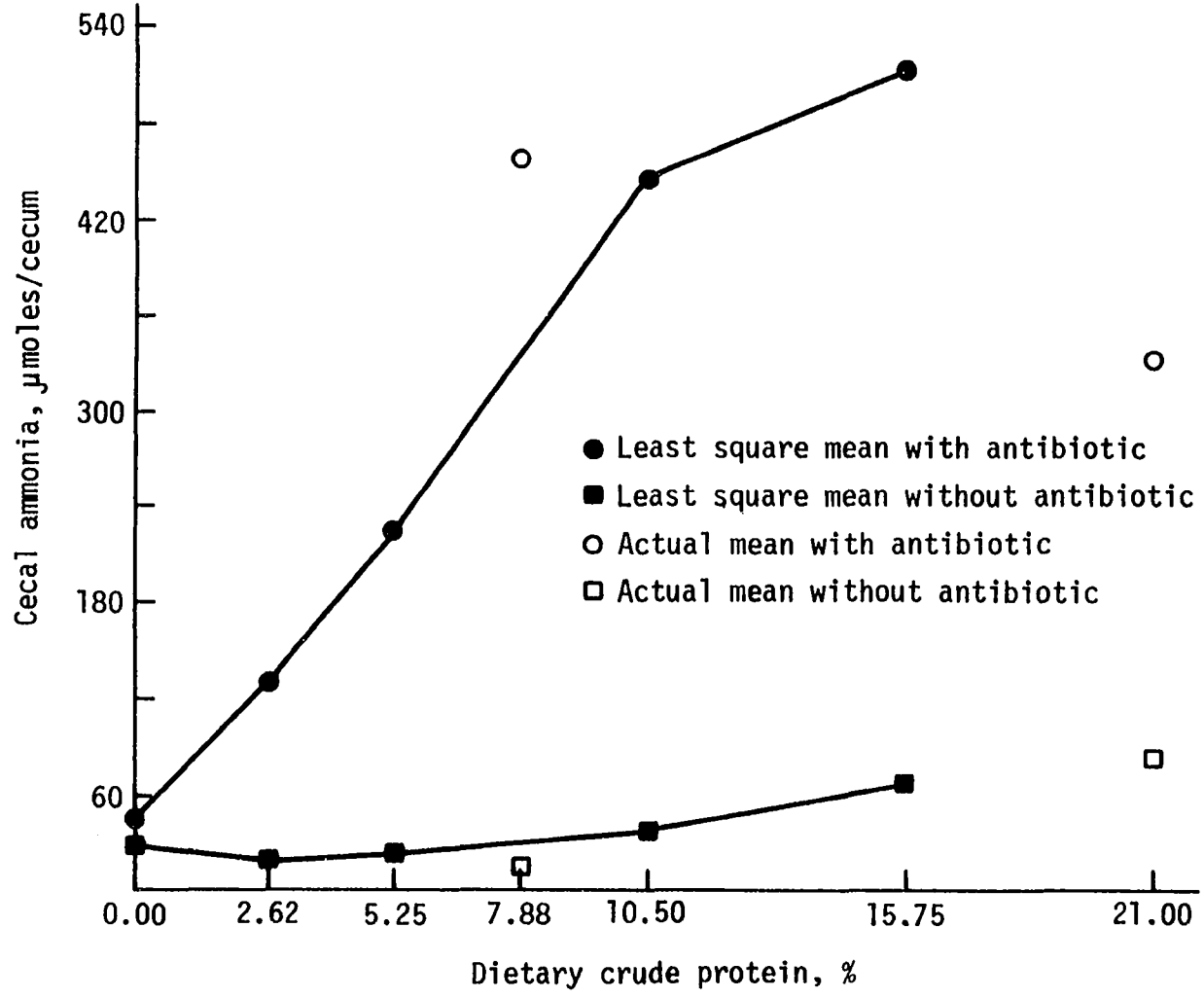

Figure 16. Effect of modified intestinal microflora on total cecal ammonia at different dietary protein levels

Cecal urea concentration was not affected by protein level (Table 21), but was increased due to antibiotic supplementation ($P<.001$) (Table 22). Individual treatment comparisons (Table 23) revealed no significant difference at 0.00% dietary protein when the intestinal microflora were modified. However, the actual urea concentration was higher with the modified intestinal microflora. The comparison of the antibiotic effect at the other levels of protein indicated that the cecal urea concentration was increased due to the antibiotics. In the ceca of rats supplemented with antibiotics there was a tendency for urea to increase as protein level increased. In the other rats cecal urea remained fairly constant. These facts, plus the relatively constant ammonia concentration in the normal microflora rats, suggested an active fermentation with the utilization of urea as a nitrogen source when antibiotics were not fed. This did not occur, or at least not to as great an extent, when the microflora were modified. Data reported by Combe and Pion (1966) and by Refat's (1978) report of Combe's work indicated that in the germfree rat urea increased in the cecum up to a concentration similar to the blood urea concentration. No urea was reportedly found in the cecal contents of conventional rats. Salter (1973) reported increased urea and uric acid losses via the feces of germfree chicks. The cecal urea concentration in the present study was somewhat higher than the aorta blood urea values of 2.93 and 3.00 μM determined in the without and with antibiotic groups, respectively. Total cecal urea, as shown in Figure 17 and Table 22, increased due to the modification of the

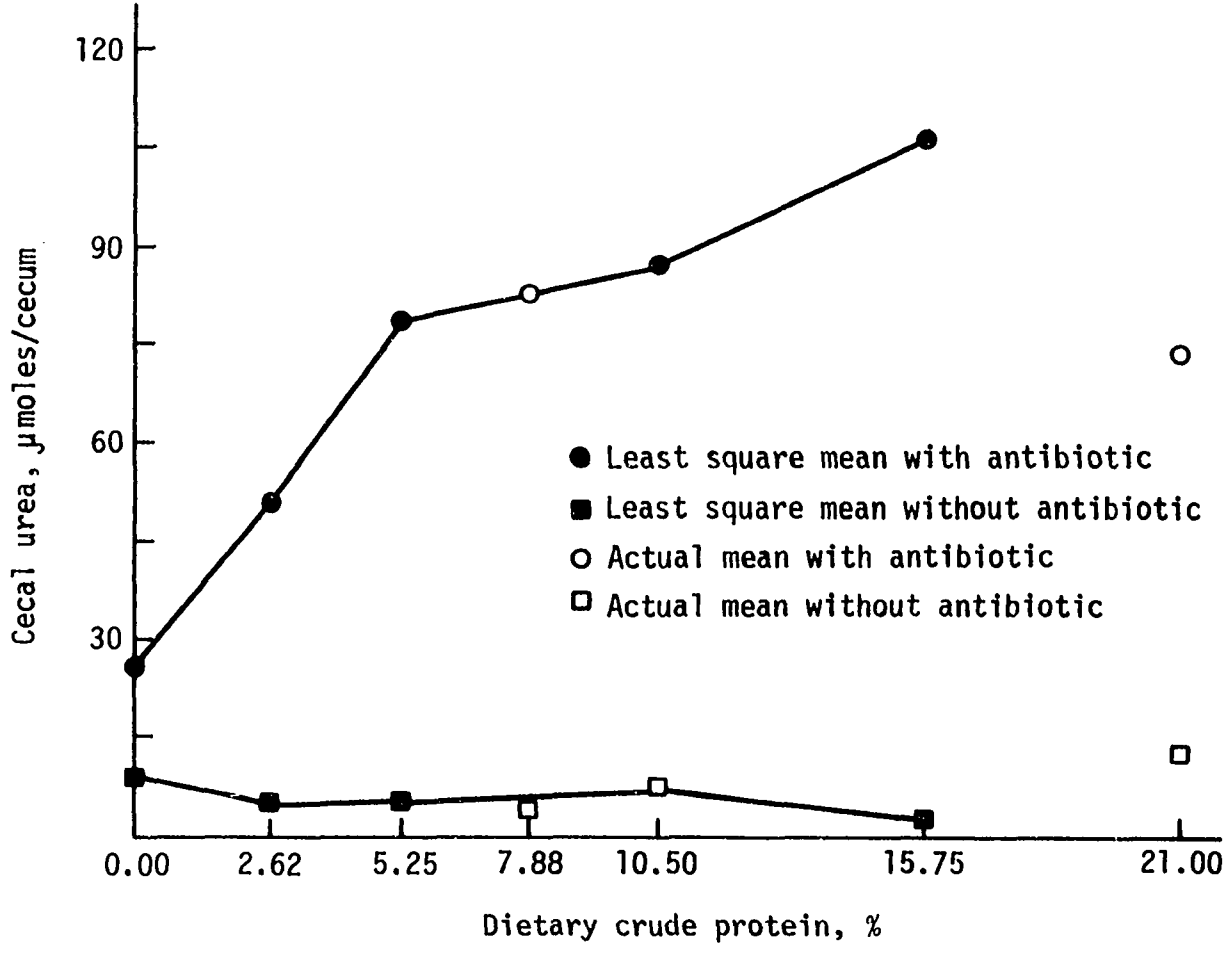

Figure 17. Effect of modified intestinal microflora on total cecal urea at different dietary protein levels

microflora (P<.001). The actual individual comparison data and statistical analyses are presented in Table A4 in the Appendix. In contrast to the effect on total cecal ammonia it appears that cecal content weight had less of an effect on total cecal urea. While the cecal content weight increased about 6.5 fold in rats fed antibiotics, the total cecal urea level was increased about 13 fold. Wostmann et al. (1973) reported that the DM content of the cecum of rats fed antibiotics could be reduced by approximately one-half. If that figure is used with the present data cecal urea would then be approximately the same on a DM basis. However, because DM could not be determined, it is not known if the total amounts are similar.

Protein level (Table 21) and antibiotic supplementation (Table 22) both increased the free amino acid level in the cecum. Concentrations of free amino acids in the cecal contents for each diet are shown in Table 23. At each protein level, except the 0.00% level, free amino acids were increased when the microbial population was modified (P<.001). The probability that the numerical difference at the 0.00% level is a true difference is 80%. Based on the free amino acid concentration differences at the other protein levels, the difference at 0.00% protein would have probably been significantly different had the experiment utilized more animals per treatment. This was the only physiological important difference, because at this level of protein the rats with the larger amount of free amino acids in the cecum also had less carcass weight loss and a greater amount of total carcass nitrogen. The normal microflora were able to keep the free amino acid level fairly constant

across all protein levels. However, when the microflora were modified, the concentration of the free amino acids in the cecum increased up to the 10.50% protein level. If the majority of the egg albumin was absorbed as would be expected, the free amino acids in the cecum had to have as their origin the intestinal endogenous protein. The increase only up to 10.50% protein would be in agreement with the data by Twombly and Meyer (1961) in which intestinal endogenous protein exhibited the largest increase with a 10% egg protein diet. The increase was not observed in rats with normal microflora in the cecum due to the microbial fermentation. Total cecal free amino acids were increased by both increasing protein (Table 21) and supplementing with antibiotics ($P<.001$) (Table 22). Figure 18 depicts this relationship graphically, with the actual data and statistical analyses included in Table A4 of the Appendix. The numerical difference at the lowest protein level has a 70% probability of being a true difference. The magnitude of the increase in total free amino acids in the ceca with antibiotics, except at the 0.00% protein level, was quite similar to the magnitude of the differences in total cecal urea. Antibiotics increased the total cecal free amino acids 17 times the amount in the cecum with a normal microflora (Table 22). Therefore, as with the total cecal urea, increases in free amino acid levels can not be totally accounted for by the increase in cecal contents. Combe et al. (1965) reported a 50 to 100 fold increase in the free amino acid content of the germfree rat's cecum.

Table 24 shows the individual comparison data for the total soluble nitrogen content in the cecum. The values were calculated by converting

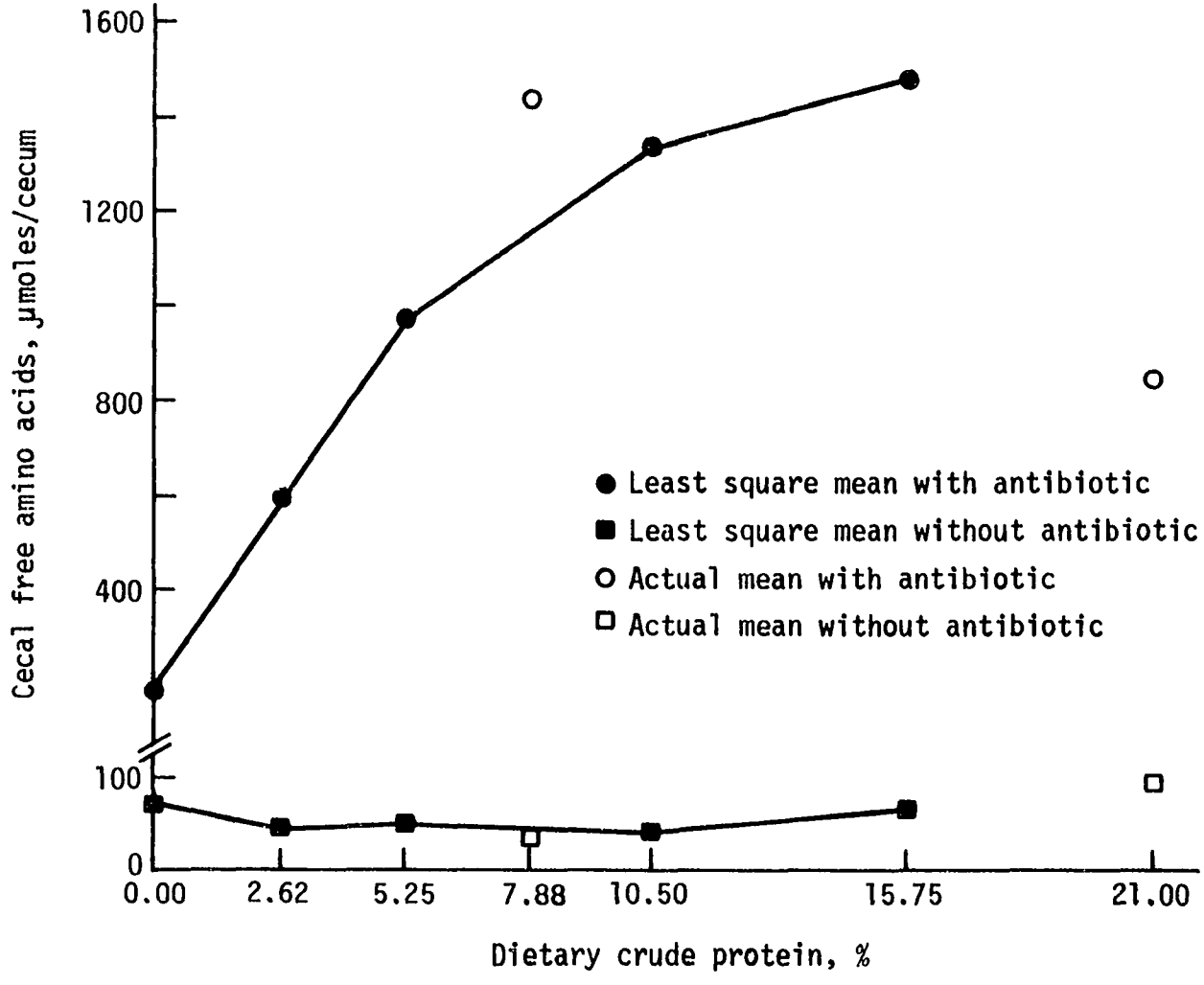

Figure 18. Effect of modified intestinal microflora on total cecal free amino acids at different dietary protein levels

the total nitrogen components discussed above to mg of nitrogen, and then adding each amount. Increasing protein in the diet with antibiotics had a significant effect on increasing total soluble cecal nitrogen. However, total soluble cecal nitrogen remained constant at all protein levels with the normal microflora. Since nitrogen retention was not affected by modification of the microflora at any protein level above the 0.00% protein diet (Table A3), it would appear that the cecal nitrogen was not effectively utilized by the animal at the higher protein levels, and thus voided. The normal microflora resulted in less cecal soluble nitrogen, and most probably the majority of the fecal nitrogen was voided as microbial nitrogen, while soluble nitrogen probably made up the largest fecal loss with modified microflora rats. This suggestion is supported by the increased fecal soluble nitrogen loss in germfree rats as reported by Combe et al. (1965) and Combe and Pion (1966).

The automated determination of urease activity could not be considered as a complete success. From preliminary assays it was determined that not only the liquid portion, but also the debris portion of each sample, should be included in the incubation with the added urea. This procedure resulted in the dialysis system becoming plugged by the debris. Subsampling the sample after incubation, with the debris flowing by gravity to the waste, alleviated the problem with the dialysis system. However, the sensitivity of the assay was greatly reduced. The sample peaks were broadened and many small peaks were

Table 24. Effect of modified intestinal microflora on total soluble cecal nitrogen at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Soluble nitrogen			
			Mean[c] mg	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<
0.00	-	5	2.59	±2.72	.30	-
	+	5	6.75	±3.34		-
2.62	-	9	1.62	±1.81	.001	.80
	+	8	19.67	±1.94		.002
5.25	-	9	1.79	±1.81	.001	.95
	+	9	32.36	±1.81		.001
7.88	-	3	1.27			
	+	3	48.85			
10.50	-	8	1.70	±1.95	.001	.95
	+	8	45.75	±1.95		.001
15.75	-	6	2.93	±2.39	.001	.70
	+	3	51.35	±3.18		.15
21.00	-	2	4.25			
	+	2	30.25			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

masked by adjacent larger ones. Because many of the original cecal samples had been completely exhausted, urease assays on all samples were not available. The following discussion is limited to 49 samples, and not all levels of protein and antibiotics were included. Therefore, statistics could only be computed on the three lower levels of protein. No tabular data will be presented due to the number of missing samples.

Values for urease activity were calculated based on ammonia production from the added urea only, and also from the added urea plus the urea already in the cecal contents. Results were similar for both calculations with lower values when based on the added urea only. Urease activity did increase as protein level increased ($P<.02$) and decreased with the modified microflora ($P<.001$) when expressed on per g of cecal content. Based on the limited statistics, it would appear that the modified intestinal microflora produced less urease at each protein level and that the activity was similar across all protein levels. However, the normal microflora tended to increase urease activity as the protein level increased. Total urease activity per cecum followed the opposite trend when antibiotic supplementation was compared, but increased at a rate comparable to the increase in cecal content weight. The exception was at the 0.00% protein level. The modified microflora did not result in a greater actual value even when expressed on a total cecum basis. The value was not significantly different from the urease activity with the normal microflora. However, with the modified intestinal microflora almost none of the added urea was hydrolyzed

when expressed per g of cecal contents, and also the total urease activity on this added urea was less than with the normal microflora. These data appear to fit the concentration values reported for ammonia and urea (Table 23). These data also suggest the possibility of a difference in urease activity at the protein level where increased nitrogen retention was observed. According to Harbers et al. (1963) and Alvares et al. (1964) the reduced urease activity would reduce any toxic effects of ammonia and increase growth.

Intestinal Nitrogen Movement and Blood Nitrogen Composition

Because the blood sampling in the first trial was somewhat variable in relation to the absorptive state of the rats, a great deal of variation was encountered. Significant trial main effects and interactions were increased when the first trial was included in the statistical analyses. Therefore, the decision was made not to use the blood values determined in the first trial. Trends were similar in the new analyses, but the variation was reduced. All blood values were somewhat similar to those of Nissen (1977) from whom this procedure was developed.

The venous flow of blood from the intestine allowed for relatively easy sampling of the blood leaving the intestine via the portal vein. Aorta samples were used to determine nitrogen composition before being influenced by the intestine. Differences in the composition of the two samples represented either output or uptake of each individual blood nitrogen component. Ammonia movement was increased with increasing

protein level (Table 25), but was not influenced by antibiotics ($P<.80$) (Table 26). Nissen (1977) indicated ammonia output remained constant as protein level increased from 10% to 30% protein. Individual treatment comparisons (Table 27) indicated no physiologically significant effects of ammonia movement.

Urea movement was not changed by protein level (Table 25) or antibiotics ($P<.70$) (Table 26). Urea movement into the blood tended to decrease without antibiotics, but increased with antibiotics when protein increased (Table 27). The difference in uptake of urea at 0.00% protein with antibiotics vs the relatively high output without antibiotics ($P<.07$) seems opposite to the expected values with a normal fermentation occurring. However, because the rats with the modified microflora retained more nitrogen, blood urea could be expected to be lower as fewer amino acids were degraded. With less urea in the blood and an increasing cecal volume, urea could be rapidly lost from the blood to the intestine with the 0.00% protein diet with antibiotics. As protein intake increased more urea entered the blood and diffused into the intestine. The normal microflora utilized this urea, and therefore, reduced output. Output was, however, increased as the modified microflora did not utilize the urea as extensively. This increased output of urea with antibiotics follows the increased urea concentration in the cecal contents (Table 23).

Free amino acid movement was not affected by protein (Table 25) or antibiotics (Table 26). Even though there was not a significant

Table 25. Effect of protein level on intestinal movement of nitrogen[a]

Dietary crude protein %	Ammonia movement[b] Mean[c] µM	SE	Significance level[d] P<	Urea movement[b] Mean[c] µM	SE	Significance level[d] P<
0.00	+0.10	±0.06	-	+0.11	±0.22	-
2.62	-0.01	±0.04	.20	+0.16	±0.14	.85
5.25	+0.06	±0.03	.25	+0.11	±0.11	.80
10.50	+0.14	±0.04	.10	+0.11	±0.12	1.00
15.75	-0.04	±0.06	.02	-0.14	±0.20	.30

Dietary crude protein %	Amino acid movement[b] Mean[c] µM	SE	Significance level[d] P<
0.00	+0.12	±0.47	-
2.62	+0.09	±0.38	1.00
5.25	+0.32	±0.26	.65
10.50	+0.83	±0.27	.20
15.75	+0.34	±0.43	.35

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Intestinal output = +, intestinal uptake = -.

[c]Least square mean ± least square standard error.

[d]Overall protein effect, but compared only to the adjacent lower protein level.

Table 26. Effect of antibiotic supplementation on blood nitrogen variables[a]

Variable	Antibiotic supplementation	Mean[b]	SE	Significance level[c] P<
Intestinal movement of:[d]				
ammonia, μM	-	0.05	±0.03	.80
	+	0.04	±0.03	
urea, μM	-	0.04	±0.10	.70
	+	0.10	±0.11	
amino acids, μM	-	0.33	±0.22	1.00
	+	0.34	±0.23	
Aorta				
ammonia, μM	-	1.34	±0.04	.50
	+	1.31	±0.04	
urea, μM	-	2.93	±0.18	.80
	+	3.00	±0.22	
amino acids, μM	-	6.44	±0.25	1.00
	+	6.42	±0.27	
Portal				
ammonia, μM	-	1.41	±0.04	.35
	+	1.35	±0.05	
urea, μM	-	3.19	±0.22	.70
	+	3.34	±0.24	
amino acids, μM	-	6.94	±0.30	.95
	+	6.97	±0.30	

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall antibiotic effect.

[d]Intestinal output = +, intestinal uptake = -.

Table 27. Effect of modified intestinal microflora on movement of nitrogenous compounds across intestine at different levels of dietary protein[a]

Dietary crude protein %	Antibiotic supplementation[b]	Ammonia movement: Number of rats	Mean[d] µM	SE	Significance level[c]: Antibiotic[e] P<	Protein[f] P<	Number of rats	Mean[d] µM
0.00	-	5	+0.09	±0.07	.95	-	5	+0.49
	+	3	+0.10	±0.10		-	2	-0.27
2.62	-	7	+0.02	±0.06	.45	.45	7	+0.25
	+	6	-0.04	±0.06	45	.25	6	+0.06
5.25	-	9	+0.05	±0.05	.90	.70	9	+0.13
	+	8	+0.06	±0.05		.25	8	+0.08
7.88	-	3	+0.14					-0.14
	+	3	+0.12					-0.10
10.50	-	8	+0.18	±0.05	.25	.07	8	+0.06
	+	8	+0.10	±0.05		.65	7	+0.16
15.75	-	4	-0.08	±0.10	.60	.03	4	-0.73
	+	3	-0.01	±0.08		.30	3	+0.45
21.00	-	1	+0.20				1	+1.79
	+	2	+0.13				2	+0.81

[a]Intestinal output = +, intestinal uptake = -.

[b]None supplemented = -, supplemented = +.

[c]Interpretation example Table 7.

[d]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[e]Antibiotic effect within each protein level.

[f]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Urea movement				Amino acid movement			
	Significance level[c]		Number of			Significance level[c]	
SE	Antibiotic[e] P<	Protein[f] P<	rats	Mean[d] µM	SE	Antibiotic[e] P<	Protein[f] P<
±0.22	.07	-	5	-0.11	±0.47	.60	-
±0.36		-	2	+0.35	±0.77		-
±0.18	.50	.45	6	+0.63	±0.53	.09	.35
±0.19		.45	5	-0.46	±0.45		.40
±0.15	.85	.65	7	+0.40	±0.38	.80	.75
±0.16		.95	8	+0.25	±0.34		.25
			3	+1.55			
			3	+1.16			
±0.16	.75	.75	6	+0.79	±0.41	.90	.50
±0.17		.75	7	+0.87	±0.37		.25
±0.31	.006	.03	4	-0.03	±0.65	.45	.30
±0.26		.40	3	+0.70	±0.55		.80
		.40	0				
			2	+1.81			

difference in free amino acid movement due to antibiotics at the 0.00% protein level (Table 27), the means indicated an output from the intestine with the modified intestinal microflora. It will be recalled that there was an indication of an increased amino acid concentration in the cecum with this diet also (Table 23). These data would tend to support the increased nitrogen retention of modified intestinal microflora rats fed the 0.00% protein diet. The uptake of amino acids due to antibiotics at the 2.62% protein level does not fit the effect at the other protein levels, but no explanation can be offered for that difference.

Based on the standard errors indicated in Table 27, it is evident that a great deal of variation exists in these data. Variation in post-absorptive states, difficulties in blood sampling, and inherent variation in the modified AutoAnalyzer procedures contributed to this variation. However, the possibility of the differences discussed being real is somewhat strengthened by significant differences in similar data of Nissen (1977). Those experiments incorporated fewer rats and more controlled procedures, and much of the above mentioned variations was reduced.

Aorta whole blood concentration values for ammonia, urea, and free amino acids were increased as protein increased (Table 28). Antibiotics had no effect on the nitrogen components determined (Table 26). At the 0.00% dietary protein level with and without antibiotics the aorta urea and free amino acid concentrations (Table 29) do not seem

Table 28. Effect of protein level on aorta and portal blood nitrogen components[a]

Dietary crude protein %	Aorta ammonia Mean[b] µM	SE	Significance level[c] P<	Aorta urea Mean[b] µM	SE	Significance level[c] P<
0.00	1.01	±0.08	-	2.54	±0.44	-
2.62	1.32	±0.06	.005	1.93	±0.31	.30
5.25	1.22	±0.05	.25	1.39	±0.24	.20
10.50	1.47	±0.05	.001	2.30	±0.25	.02
15.75	1.60	±0.07	.15	6.65	±0.36	.001

Dietary crude protein %	Portal ammonia Mean[b] µM	SE	Significance level[c] P<	Portal urea Mean[b] µM	SE	Significance level[c] P<
0.00	1.11	±0.09	-	3.34	±0.48	-
2.62	1.31	±0.06	.07	2.09	±0.33	.04
5.25	1.28	±0.05	.70	1.50	±0.26	.20
10.50	1.60	±0.05	.001	2.45	±0.28	.02
15.75	1.58	±0.09	.80	6.94	±0.48	.001

[a]Protein level 7.88 and 21.00 not included since not included in overall analysis.

[b]Least square mean ± least square standard error.

[c]Overall protein effect, but compared only to the adjacent lower protein level.

Aorta amino acids		
Mean[b] µM	SE	Significance level[c] P<
5.18	±0.55	-
6.03	±0.49	.30
6.33	±0.34	.65
7.77	±0.34	.005
6.85	±0.44	.15

Portal amino acids		
Mean[b] µM	SE	Significance level[c] P<
5.93	±0.59	-
6.11	±0.53	.85
6.66	±0.32	.40
8.53	±0.35	.003
7.54	±0.59	.20

to agree with the cecal concentrations of these compounds (Table 23). The increase observed in urea at the 10.50% protein level with either antibiotic supplementation indicated the protein supply exceeded the need for amino acids, and amino acids were deaminated with a resultant increase in urea. Statistical analysis of only the one trial in which the 7.88% diet was used indicated the increase occurred between the 7.88% and 10.50% diet. No explanation can be offered for other differences observed in aorta nitrogen components.

Portal whole blood nitrogen values are presented in Tables 26, 28, and 30. As with aorta nitrogen, increasing protein increased portal ammonia, urea, and free amino acids (Table 28), but again antibiotics had no effect (Table 26). Individual treatment comparisons (Table 30) revealed no physiologically significant effects with the possible exception of greater urea concentrations at the higher protein levels with antibiotics. This may be an indication of less urea hydrolysis in the intestine with the modified microflora.

Initial Slaughter Group Comparison

Comparisons between the initial slaughter rats and rats fed one of the three lowest protein diets were used to determine the protein level at which no change occurred in relation to the estimated starting value. The variables included in the analysis, the initial slaughter means, and the probability that the means were different from the

Table 29. Effect of modified intestinal microflora on aorta whole blood nitrogen components at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Aorta ammonia: Number of rats	Mean[c] µM	SE	Significance level[b]: Antibiotic[d] P<	Protein[e] P<	Number of rats	Mean[c] µM
0.00	-	5	1.14	±0.10	.08	-	5	3.35
	+	5	0.89	±0.12		-	4	1.74
2.62	-	7	1.39	±0.08	.25	.06	7	1.96
	+	6	1.25	±0.09		.02	6	1.90
5.25	-	9	1.22	±0.07	.90	.15	9	1.33
	+	8	1.23	±0.07		.90	8	1.46
7.88	-	3	1.51				3	1.77
	+	3	1.46				3	1.51
10.50	-	8	1.44	±0.07	.70	.03	8	2.17
	+	8	1.49	±0.07		.02	8	2.44
15.75	-	6	1.53	±0.09	.35	.50	6	5.85
	+	3	1.67	±0.12		.20	3	7.47
21.00	-	1	1.15				1	1.88
	+	2	1.84				2	12.83

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Aorta urea			Aorta amino acids				
	Significance level[b]		Number of			Significance level[b]	
SE	Antibiotic[d] P<	Protein[e] P<	rats	Mean[c] µM	SE	Antibiotic[d] P<	Protein[e] P<
±0.49	.03	-	5	5.71	±0.60	.25	-
±0.63		-	4	4.66	±0.78		-
±0.40	.95	.04	6	5.71	±0.68	.45	1.00
±0.43		.85	5	6.35	±0.58		.10
±0.33	.80	.25	7	6.42	±0.49	.80	.40
±0.35		.45	8	6.24	±0.44		.90
			3	6.05			
			3	6.54			
±0.35	.60	.09	7	7.52	±0.49	.50	.15
±0.35		.06	7	8.01	±0.46		.009
±0.43	.03	.001	6	6.86	±0.53	.95	.40
±0.57		.001	3	6.84	±0.71		.20
			0				
			2	8.88			

Table 30. Effect of modified intestinal microflora on portal whole blood nitrogen components at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Portal ammonia: Number of rats	Mean[c] µM	SE	Significance level[b]: Antibiotic[d] P<	Protein[e] P<	Number of rats	Mean[c] µM
0.00	-	5	1.23	±0.10	.20	-	5	4.09
	+	3	0.99	±0.14		-	3	2.59
2.62	-	7	1.41	±0.08	.08	.15	7	2.21
	+	6	1.21	±0.08		.20	6	1.97
5.25	-	9	1.27	±0.07	.85	.20	9	1.46
	+	8	1.29	±0.07		.50	8	1.54
7.88	-	3	1.65				3	1.63
	+	3	1.58				3	1.41
10.50	-	8	1.63	±0.07	.70	.001	8	2.23
	+	8	1.58	±0.07		.005	7	2.67
15.75	-	4	1.49	±0.13	.35	.40	4	5.97
	+	3	1.66	±0.11		.60	3	7.92
21.00	-	1	1.35				1	3.67
	+	2	1.97				2	13.64

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Portal urea			Portal amino acids				
	Significance level[b]		Number of			Significance level[b]	
SE	Antibiotic[d] P<	Protein[e] P<	rats	Mean[c] µM	SE	Antibiotic[d] P<	Protein[e] P<
±0.52	.15	-	5	5.65	±0.65	.65	-
±0.76		-	3	6.20	±0.95		-
±0.43	.70	.008	6	6.34	±0.73	.65	.50
±0.46		.50	5	5.89	±0.62		.80
±0.35	.90	.20	9	6.83	±0.44	.65	.60
±0.38		.50	8	6.49	±0.47		.45
			3	7.60			
			3	7.70			
±0.38	.45	.15	7	8.32	±0.50	.55	.04
±0.41		.05	8	8.75	±0.47		.002
±0.73	.05	.001	4	7.55	±0.90	1.00	.50
±0.61		.001	3	7.54	±0.76		.20
			1	8.19			
			2	10.68			

six lowest protein diets are all included in Table 31. Also, a table number is provided for easy reference to actual data for the diet means.

As would be expected, initial weight was not different, but final weight was different in the treatments compared to the initial slaughter group's final weight. The exception was the 2.62% protein diet without antibiotics. It can be speculated that had the cecal weight of the rats on the corresponding diet with antibiotics not been increased, the final weight would have been similar to that of the initial slaughter rats. Similar carcass weights tend to support this conclusion.

Even though carcass nitrogen concentration was lower ($P<.001$) in the initial slaughter group as compared to all other groups, total carcass nitrogen was similar to the rats fed the 2.62% protein diet. Total carcass ammonia was higher in the initial slaughter group than in the 0.00% protein group, but was similar to the concentration in the other two groups. However, total carcass urea was higher in the initial slaughter group. This may be a reflection of the physiological processes due to length of time without feed when the animals were initially weighed, stratified, assigned to cages, and the experiment started. Therefore, there was some time delay in sacrificing the initial slaughter animals. If this situation increased carcass urea concentration due to protein degradation it might have led to the decreased carcass nitrogen concentration observed in the initial

Table 31. Initial slaughter group variables comparison to variables in the three lowest protein groups

Variables	IS[a] Mean[b]		SE
Initial weight, g	122.75	±	1.40
Final weight, g	122.75	±	1.92
Carcass weight, g	110.87	±	1.93
Carcass nitrogen, wet basis, %	3.15	±	0.03
Total carcass nitrogen, g	3.49	±	0.06
Total carcass ammonia, mM	3.53	±	0.33
Total carcass urea, mM	0.61	±	0.05

[a]IS = initial slaughter group, 12 rats.

[b]Least square mean ± least square standard error.

Significance level from diet:						Diet data in table
0.00		2.62		5.25		
-	+	-	+	-	+	no.
.65	.10	.30	.25	.20	.30	-
.001	.001	.65	.04	.001	.001	A1
.001	.001	.08	.09	.001	.001	A2
.001	.001	.001	.001	.002	.001	A2
.001	.001	.35	.40	.001	.001	A2
.04	.009	.09	.75	.75	.95	10
.002	.002	.001	.001	.003	.001	10

slaughter group. However, the magnitude of difference seems too great to be explained in such a manner.

GENERAL DISCUSSION

Significance of Results

As previously discussed, MFN losses are considered by most nutritionists to be a maintenance nitrogen need, even though it is more correctly an expense related to the digestive processes. The use of MFN primarily has been as a portion of the biological value determination for different proteins. Few diets are actually balanced on the basis of biological value. Biological values are used primarily as a ranking tool in which the relative quality of proteins or diets can be compared. The nitrogen (amino acids) needed to cover the expense of digestion (MFN) is subtracted from the fecal nitrogen voided by the test animal; in effect this credits the diet for covering this loss. H. H. Mitchell contributed to the development of the biological value concept, and along with his coworkers, students, and former students conducted the majority of the research concerning MFN. Mitchell (1926) indicated the MFN value of 2 mg per g DM was somewhat constant across all species fed diets not containing excess indigestible carbohydrates. That value has become widely accepted for use in monogastric animals. However, MFN in ruminants is usually reported to be 2 to 3 times greater, and most nutritionists ascribed this fact to the abrasive properties of the larger amounts of roughage consumed.

New knowledge in the area of rumen metabolism has led to the development of concepts more exacting in their assessment of the

protein nutrition of the ruminant. Not only did these new systems improve concepts of nitrogen utilization by ruminal bacteria, but they also stimulated interest in the fermentation occurring in other portions of the digestive tract. In order to develop a complete scheme certain of these new systems required MFN values.

Newer concepts of ruminal fermentation activities and changes in fecal nitrogen excretion under certain circumstances seem to point to an active lower intestinal fermentation in all animals. It is proposed that this fermentation has a definite effect on the excretion of the MFN. Since the lower intestinal fermentation was not adequately understood at the time of Mitchell's development of the MFN concepts, the value presented above is probably higher than the true MFN excretion occurring with practical diets. The basis for this statement will be presented in subsequent discussions.

The work of Nasset (1957), Geiger et al. (1958), Nasset and Ju (1961), Twombly and Meyer (1961), and Zebrowska and Buraczewska (1972a,b) point out the importance of the intestinal endogenous nitrogen because substantial quantities are secreted into the intestine. If the dilution of exogenous nitrogen by endogenous intestinal nitrogen is one to one (Nasset, 1957; Twombly and Meyer, 1961), not all the intestinal endogenous nitrogen can be excreted, or there would be no net protein accretion in the body. Fauconneau and Michel (1970) and Refat et al. (1977) pointed out the importance of the intestinal endogenous nitrogen in correcting the amino acid deficiencies of the exogenous protein.

However, Crompton and Nesheim (1969) and Fauconneau and Michel (1970) indicated the exogenous amino acids were digested and absorbed more rapidly than the intestinal endogenous nitrogen. The intestinal endogenous nitrogen must then be digested and reabsorbed in the lower regions of the digestive tract.

Because the bacterial population increases in the lower regions of the digestive tract, the possibility of the intestinal endogenous nitrogen being fermented by the bacteria is increased. The nitrogen of the intestinal endogenous amino acids could be deaminated, and the ammonia lost via the urine. Also, the ammonia could be incorporated into microbial protein, and the microbial cells voided in the feces. In either case this would result in a substantial loss of preformed body nitrogen, especially when zero or low protein diets are fed as is done when determining MFN.

Research such as that reported by Percora (1953) and Harmon et al. (1968) indicates that the microflora in the lower intestine of the monogastric animal influences fecal nitrogen losses and affects nitrogen retention. In the former report MFN was decreased by 21% when the intestinal microflora were modified with antibiotics. Carcass weight was also increased with the antibiotic supplemented rats indirectly indicating a possible increase in nitrogen retention. The researchers in the latter reference reported a MFN reduction of 61% when comparing germfree rats to conventional rats fed low levels of egg protein. Extrapolation of the fecal nitrogen values, when increasing dietary protein was fed, to zero protein intake resulted in a MFN value

of 0.03 mg per g DM intake. Therefore, the intestinal endogenous protein must have been reabsorbed and reutilized and not excreted as occurred in the conventional animal. Pion et al. (1977) indicated apparent nitrogen digestibility decreased when a highly digestible protein was fed to conventional animals as compared to germfree animals. It can be postulated that this effect is due to microbial scavenging in the large intestine of the intestinal endogenous nitrogen. Most, if not all, of the microbial protein produced would be excreted in the feces. Therefore, in a germfree animal, and to a certain extent in animals with antibiotic modified intestinal microflora, less intestinal endogenous nitrogen would be lost. The MFN values would then be decreased.

Although included in the original experimental procedure, the present research did not allow for the determination of MFN directly. However, the experimental conditions were similar to those in the work by Harmon et al. (1968) except the normal intestinal microflora were modified by antibiotic supplementation. At the 0.00% dietary protein level the modification of the intestinal microflora allowed greater carcass nitrogen retention (Figure 13). This increase in carcass nitrogen was not due to increases in the urea or ammonia concentration in the carcass. Therefore, based on the results of the present study and those of Harmon et al. (1968), it was concluded that the MFN loss was reduced in the present study. Chawla et al. (1976) reported similar data except that weight gain, corrected for cecal size, was increased when antibiotics were supplemented with a zero casein diet. It has generally been

accepted that antibiotics improve performance especially at low dietary portein levels and with lower quality proteins. However, the exact mechanism has been the source of considerable debate (Visek, 1978). Based on the data of the present study and on research available in the literature, it is proposed that the increase in carcass gain, carcass nitrogen, nitrogen retention, and/or growth with antibiotics at lower intakes of protein occurs due to the increased amino acids available for reabsorption and reutilization. With a normal microflora, these amino acids are rapidly destroyed by the microbial fermentation (Allison, 1970; Hecker, 1971b; Chalupa, 1975).

If this concept is correct, there should be differences in the cecal and fecal composition. Mason (1969) indicated that in conventional animals bacterial nitrogen can be 97 to 100% of the total fecal nitrogen. Also, the MFN was almost entirely microbial nitrogen (Mason et al., 1976) in conventional animals. Fecal nitrogen composition of germfree animals is composed of greater quantities of nonprotein nitrogen. Also, with the possible exception of the chicken, germfree and antibiotic modified intestine microflora animals excrete less fecal nitrogen. In germfree and antibiotic modified animals cecal total nitrogen is increased mainly due to an increase in soluble nitrogen (Combe et al., 1965; Combe and Pion, 1966). Free amino acids were also substantially increased. This effect was also observed in the present study. The total amount of cecal free amino acids, their concentration, and the total amount of cecal soluble nitrogen were significantly

increased with the modified intestinal microflora. The values with 0.00% dietary protein diets were not significantly different, but the trend was indicative of the increase observed with the other diets. Combe and Pion (1966) reported an increased concentration of urea and decreased concentration of ammonia in the germfree cecum. In the pig similar effects were observed when antibiotics were fed (Henderickx and Decuypere, 1973; Vervaeke et al., 1976; Deguchi et al., 1978). Antibiotic supplementation of the rats used in the present study resulted in no difference in cecal ammonia concentrations at each protein level. Cecal urea concentration was reduced with the normal intestinal microflora.

The free amino acid concentration in the cecal contents of the rats fed the 0.00% protein diet in the present study is of utmost importance to the concepts proposed in this dissertation. According to Kelleher and Bruckner (1977), absorption should not have been impaired in the rats with the modified intestinal microflora. Greater amounts of free amino acids would be expected without the normal fermentation occurring, and the present data seem to substantiate this effect. However, if the free amino acid concentration is the same with and without antibiotics, it may then be an indication of increased absorption in the intestine when the body's amino acid demand is high. This could also occur in the rats with the normal microflora, but the microbes would ferment most of the amino acid nitrogen before absorption could occur. Either effect would explain the difference in carcass nitrogen retention in the rats

fed the 0.00% protein diets with and without antibiotics. However, no increase in nitrogen retention was observed at the other protein levels even with increased cecal free amino acid levels in the antibiotic fed rats. It is possible that the effect seen with the 0.00% protein diets was due to a methionine-microbial interaction as discussed in the Literature Review and later in the General Discussion. Once egg protein is included in the diet and intestinal endogenous nitrogen is increased, the host's methionine requirement is met, and no additional retention occurs. Also, it is possible that the increase in cecal urea, with increasing dietary protein, may provide the needed nitrogen for the microbes, and that some amino acids are spared. If similar quality and quantity of amino acids are absorbed, nitrogen retention would be relatively constant. However, the remaining amino acids in the intestine would be deaminated in the cecum containing normal microflora, but allowed to accumulate with a modified microflora in the cecum.

The data presented from the present study may also indicate that under the condition encountered, such as less total intake, intake by intubation, readily digestible energy sources, and antibiotics, the microfloral population of the intestine was severely modified at the 0.00% level due to a reduction in required nutrients and the supplementation of antibiotics. At the higher levels of both total intake and protein intake the modification may not have been as severe. Therefore, the nitrogen retention response was limited to that one level of protein. However, the results would still indicate the effect of the microbial population on fecal nitrogen excretion and on the host's protein

nutrition, especially at the protein levels where MFN is normally determined.

Reports by Combe et al. (1965), Schaedler (1973), and Visek (1978) suggested a substantial (30 to 40%) increase in replacement of intestinal mucosal cells in the conventional animal vs the germfree or modified intestinal microflora animal. Also, there were less mucosal cells in the germfree (Combe et al., 1965) as determined by DNA content of the intestine. In contrast, Levenson and Seifter (1974) reported similar turnover of intestinal cells. If the mucosal turnover is reduced, then there would be less preformed protein sloughed into the intestine, and a reduction in the intestinal endogenous nitrogen in the intestine of these animals. According to Visek (1972), a 100 g conventional rat needs to replace one g of intestinal cells per day. If the growth rate is five g per day, then one additional g or 20% of the weight gain is needed just to replace lost intestinal cells. If that loss is reduced by 40% or 0.4 g and the spared nutrients used for additional growth, the rat should increase his weight gain by 8%. This projected improvement is within the range of growth rate improvement reported in the literature between germfree or antibiotic supplemented animals and conventional animals. The total carcass nitrogen with the modified intestinal microflora in the present study was 11% greater than found with rats having the normal microflora. These data imply that a reduction of mucosal turnover may reduce the intestinal endogenous nitrogen that would be exposed to any microbial fermentation occurring in the digestive tract. This fact should be considered to be a part of the concepts proposed above.

Because the end result is the same, both can be considered to complement each other.

Therefore, it appears that in conventional animals fed low or zero nitrogen diets or diets containing highly digestible and utilizable proteins, the microflora of the entire tract are deprived of dietary nitrogen. Due to their location in the gut there is ample opportunity for destruction of the only nitrogen available, intestinal endogenous nitrogen. Once incorporated into microbial protein, the nitrogen originally from the host's body proper is excreted in the feces, and the microbes have essentially "robbed" the host. In a germfree animal or animal supplemented with antibiotics a different situation exists. The host is not competing with the microbes in the prime absorptive region, the small intestine. Neither is there the problem of an active microbial fermentation in the large intestine. Without the intestinal microbes or with a modified intestinal microflora, the intestinal endogenous proteins have more opportunity to undergo hydrolysis, and the amino acids may be absorbed. However, most nutritionists consider the only absorption occurring in the large intestine to be water and water soluble compounds along a concentration gradient. The literature does not present any clear trend as to the absorbability of these amino acids in the lower intestine. Research by Herskovic et al. (1967), Demaux and coworkers (Ulyatt et al., 1975), Yang et al. (1972), Ben-Ghedalia et al. (1976), James and Smith (1976), and Kelleher and Bruckner (1977) can be used to point out the possibility of amino acid absorption in the

lower intestine. In contrast, the research by Elliott and Little (1977), Mason et al. (1977), and Zebrowska et al. (1978b) in which intact protein was infused into the cecum resulted in no useful absorption of the nitrogen. Cecal content concentration and movement of amino acids from the intestine to the blood in the present study indicate the possibility that more amino acids were available for utilization by the host animal when the intestinal microflora was modified. Absorption in the lower small intestine and in the large intestine needs to be critically evaluated with low protein diets in future research. However, absorption in the large intestine may not be a necessity for the concepts presented to be valid. Additional absorption in the lower small intestine may be sufficient to result in the effect observed.

Besides the basic effect on fecal nitrogen due to the microbial fermentation proposed above, certain other factors can influence the losses due to the fermentation. Salter et al. (1979) indicated that with ruminal bacteria fermentations methionine may be a limiting factor for microbial growth on low protein diets. The data seem to indicate methionine is needed in a preformed state for effective microbial growth. If preformed methionine is also needed by the lower intestinal microbes, this fact could influence the fecal nitrogen losses. It is known that methionine is one of the most tightly bound amino acids to the absorptive systems in the gut (Reiser and Christiansen, 1965). The data of Salter and Fulford (1974) indicated the intestinal endogenous protein may be limiting in methionine. Therefore, on a

protein free diet less methionine would be found in the gut due to the limited quantity in intestinal endogenous protein and to the host's intestinal absorptive systems. Intestinal endogenous methionine would then become scarce as the microbes digest it. Doft and McDaniel, as reported by Levenson and Seifter (1974), proposed a similar concept when they found that 40%, 50%, and 50% of the supplemented methionine, threonine and tryptophan, respectively, were destroyed by the intestinal microbes when protein free diets were fed. Along with a lower intestinal methionine concentration, less plasma sulfate would also be available, and therefore, due to both factors, the intestinal microbes would be limiting in sulfur for microbial protein synthesis. This is somewhat in contrast to the balancing effect of intestinal endogenous protein as proposed by Refat et al. (1977).

Indirect support of this proposal can be found in work done with supplemental sulfur amino acids. Ackerson and Blish (1925) found that cystine "exerted a protein sparing effect out of proportion to its nitrogen content." The birds were fed a protein free diet, and the endogenous urinary nitrogen loss was reduced by 43% with cystine supplementation. Similarly, Allison et al. (1947) found that methionine depressed urinary nitrogen excretion in dogs fed protein free diets. The decline in urinary nitrogen was 26% with fecal nitrogen remaining the same. Approximately the same percentage decline was noted when egg albumin was supplemented with methionine, but in that experiment fecal nitrogen declined also. Experiments conducted by Brush et al. (1947) with rats also demonstrated the protein sparing effect of

methionine. In an extensive series of experiments methionine consistently reduced urinary nitrogen loss and, to a lesser extent, fecal nitrogen loss. The other essential amino acids were at best only about two-thirds as effective as methionine.

More recently, Japanese workers have demonstrated that supplemental methionine and threonine in a protein free diet can reduce weight loss in rats by as much as 1.20 g per day (Yokogoshi et al., 1974; Yokogoshi et al., 1977). Supplementation also reduced urinary nitrogen loss, with urea the urinary nitrogen component most reduced. However, amino acid catabolizing enzymes were not depressed in the liver. Aspartic acid, glutamic acid, alanine, and serine concentrations were reduced in the liver. Because glutamic acid, serine, and alanine are involved in many transaminations and because aspartic acid is a required substrate for urea synthesis, any reduction in the concentration of those amino acids could reduce nitrogen shuttles in the liver. Also proposed was a more effective utilization of body soft tissue amino acids with methionine and threonine supplementation.

Based on these reports, the intestinal endogenous protein (amino acids) is proposed to be more effectively utilized. Hair, feathers, wool, and hoofs are all rich in sulfur amino acids (Harper, 1975). As these tissues are lost from the body there is normally no way to recapture the sulfur amino acids lost with these tissues. Therefore, replacement of these tissues will result in a deficiency in sulfur amino acids. The sulfur amino acids will be mobilized from the soft tissue

to be used for keratin production (Mitchell, 1962). If this occurs, the remainder of the soft tissue's amino acids, which were also released, will be transported to the liver, deaminated, and urea synthesized. This urea can be lost via the urine, or more importantly as discussed above, lost via the feces as microbial protein. Therefore, if the intestinal microbes are "robbing" the host of intestinal endogenous sulfur already, this aggravates the tissue loss, and a vicious cycle is initiated.

Supplementation of methionine can supply this need of the microbes and/or host and reduce urea production and excretion as seen in the research reported above. Support for this proposal can be found in the research of Johnson et al. (1947) in which men on low protein intakes did not respond to methionine supplementation. It is fairly evident that the keratin production in man would be less than in animals completely covered with hair, wool, or feathers and having large hoofs. Additionally, Schaedler (1973) indicated that germfree mice can gain weight when fed corn as the sole food while the conventional animals lose weight. It is known that corn is low in methionine, and therefore, the amino acids of corn plus the spared amino acids of intestinal endogenous protein, especially methionine, can complement each other. This principle is similar to work being done in feedlot ruminants in which the expensive protein source, with its methionine, is protected so as to bypass the rumen, but complements the other dietary protein plus the ruminal microbial protein in the small intestine (Thomas, 1977).

Fermentable energy presented to the fermentation occurring in the lower intestine can also influence fecal nitrogen excretion. Evidence of an increased lower intestinal fermentation effect on nitrogen loss has been presented by Thornton et al. (1970). These researchers demonstrated the movement of urea from the urine, and its excretion in the feces as the cecal fermentation was stimulated by glucose (Figure 1) without altering nitrogen balance.

Also, calculation of the ratio of μg 2,6-diaminopimelic acid to mg fecal nitrogen from the data of Mason and Palmer (1973) produces essentially a constancy across all diets. This indicates similar amounts of bacterial cell wall per unit of fecal nitrogen and suggests that nitrogen limits the large intestine fermentation when a highly digestible protein is fed with raw potato starch. MFN with the raw potato starch diets averaged 4.09 mg per g DM intake. This is approximately twice the amount voided in rats fed diets containing low amounts of fermentable energy. More importantly, these values approach the MFN values observed in ruminants. Therefore, on a normal diet with substantial fermentable energy reaching the large intestine the ruminant animal may excrete more MFN than would be excreted without the fermentable energy. If ruminal bypass and microbial protein are 90% and 80% digestible, respectively, in the small intestine (Burroughs et al., 1975; Mason and Frederiksen, 1979), it is proposed that intestinal endogenous protein is used to cover the majority of the nitrogen requirement of the increased fermentation, especially at low

nitrogen intakes. This excretion of nitrogen originating from body α amino nitrogen would then need to be replaced by dietary nitrogen.

Based on literature reports and the results of the present study, it is proposed that the lower intestinal microbes influence fecal nitrogen excretion at low nitrogen intakes. The basic loss of nitrogen due to the fermentation can be influenced by methionine and fermentable energy in the lower intestine. Any aggravation of the host's and/or its microflora's protein metabolism at these low protein intakes resulting in an increased fermentation in the large intestine would increase the MFN value. At these low protein intakes a possible sequence of events is proposed to occur:

1) recycled urea reduced,
2) intestinal endogenous nitrogen degradation increased,
3) increased loss of sulfur amino acids from the host to the microbes,
4) host body tissue protein mobilized to supply sulfur amino acids for critical tissues,
5) amino acid balance upset in host,
6) urea production increased,
7) intestinal microbial production increased,
8) nitrogen excretion increased, and

the cycle repeats and begins to perpetuate itself. If this concept is true, one of the most economically important ramifications would be in the beef cow subsisting on low protein, high fiber pastures. If the MFN

loss in ruminants is increased due to the large intestine fermentation by 4 to 6 mg per g DM intake above the true MFN loss, then every kg of pasture taken in may actually cost the cow 25 to 40 g (4 to 6 g fecal nitrogen X 6.25) of protein.

However, a different situation is proposed to occur at higher levels of protein feeding as would be normally encountered in most practical situations. A more detailed interpretation of the many literature reports of nitrogen balance at higher levels of nitrogen feeding results in the suggestion that the loss of nitrogen is not as severe as when determined at a low level of nitrogen intake (Figures 2 to 6). The results of the present study are similar to those results when total carcass nitrogen was plotted against nitrogen intake (Figure 12). The line predicted by the equation did not pass through the value determined for the initial slaughter group's total carcass nitrogen, and therefore, does not fit exactly the other graphical data mentioned. However, it is felt that similarity between the predicted value determined by the regression equation and the actual value when rats were fed 0.00% protein with antibiotics indicates a similarity to any predicted values at zero nitrogen intake in Figures 2 to 6. Because all the data in those graphs tend to approach the origin at zero nitrogen intake, it can be suggested that the maintenance nitrogen requirement may not be the same at both high and low intakes of nitrogen.

These literature reports plus the data of the present study indicate that at most practical levels of protein feeding the origin of the MFN

may not be the body proper as has been commonly accepted. At these practical protein feeding levels microbial protein makes up a large portion of the nitrogen voided in the feces (Mason et al., 1976: Mason, 1979). According to the work by Nolan and coworkers (Nolan and Leng, 1972; Nolan et al., 1973; Nolan et al., 1976), the majority of the urea hydrolysis in the ruminant occurred outside the rumen and would contribute nitrogen to the fermentation in the lower intestine. Nitrogen needed by the microbes to satisfy their nitrogen requirement would then be provided and would allow for a sparing of intestinal endogenous nitrogen.

Support for this proposal is provided in recent research with germfree and conventional mice (Yamanaka et al., 1977) and with germfree and SPF pigs (Deguchi et al., 1978) indicating greater growth rates in the conventional animals. However, the animals were fed a high protein diet, 10% CP from egg protein in the mouse diets and 22% CP from milk in the pig diets. Therefore, according to the above mentioned concept, the conventional animal could be expected to be at least equal to the germfree animal in growth rate at these protein levels. The isolation environment for the germfree animals could also be a possible reason for the lowered growth rate. The data of Yamanaka et al. (1977) indicated that total carcass nitrogen was higher in conventional mice, but carcass nitrogen per 10 g body weight gain was higher in germfree mice. Additionally, nitrogen retention by a slaughter technique was similar for both groups. No information was provided as to the genetics of the mice, and therefore, the conventional mice

may have had the potential for faster gains, and the ad libitum intake procedure allowed them to outgain the germfree mice while nitrogen retention per unit of food intake was not different. The greater energy retention tends to support this suggestion. It appears that in these experiments with high levels of protein intake, the recycled urea in the large intestine spared preformed nitrogen for reutilization.

Therefore, the above data from the literature and those of the present study indicate that urea, which is already a waste product, may be recycled to the gut and satisfy the intestinal microbial nitrogen requirement. It is proposed that the intestinal endogenous nitrogen is then reutilized. Therefore, at these higher protein feeding levels the true maintenance protein requirement would become quite small. Because urea is a waste product and appears to be preferentially voided via the feces (Thornton et al., 1970) as microbial protein, the microbial protein portion of the MFN should not be charged as an expense of digestion to the feed. If this concept of greater intestinal endogenous protein loss at low protein intakes and very little at high protein intakes is true, it will require a complete reevaluation of biological values of proteins.

Results of biological value calculations based on the carcass nitrogen values determined in the present study (Figures 14 and 15) indicate that MFN as it is normally determined should not be included in biological value calculations. The biological value computed for the diets without antibiotics decreased at each higher level of protein, and the decrease was of the same magnitude both above and below the diet

at which nitrogen retention was maximal, the 10.50% protein diet. Above 10.50% protein with antibiotics the decrease was also similar. However, below the protein level for maximal nitrogen retention, the biological values, determined in rats with modified intestinal microflora, were similar as they were based on less nitrogen loss when protein free diets were fed. When this same nitrogen loss is applied to the rats with the normal microflora, the new biological value also becomes constant below 10.50% protein. It is more logical that biological value would be constant at all levels of intake up to the maximum retention of nitrogen. However, with the intestinal microbes incorporating intestinal endogenous nitrogen into microbial nitrogen at low protein intakes this body nitrogen is then used to credit the dietary protein for its ability to cover the replacement of the intestinal endogenous protein. Technically this is correct at low levels of nitrogen intake. But if biological value, or any protein evaluation is to be of general usefulness, it should have the greatest application and meaning with practical feeding situations. Therefore, use of a MFN value determined with a protein free diet or a diet containing a highly utilizable protein can not be extrapolated to a practical situation where the MFN is mainly comprised of nitrogen originating from a waste product, urea.

It is then proposed that the use of MFN be discontinued from use in the determination of protein evaluation at normal protein feeding levels. Roy et al. (1977) reporting as the Protein Sub-Group of the Agricultural Research Council Working Party on Nutrient Requirements

of Ruminants expressed a similar concern. A new system was proposed by that group since the concern described above "led to a lack of confidence in systems. . .requiring an estimate of metabolic faecal N." The new system did not include MFN as a portion of the protein requirement deserving consideration. Use of the "normal" ruminant values in the MP system (Burroughs et al., 1974) would have overestimated the nitrogen needed to cover the expense of digestion. According to the above discussion, the MFN value should be even lower than the 2 mg per g DM intake used. However, such a small amount in a system still refining its values acts as a safeguard. As the MP system is fully developed with more exact values, it might be advisable to reduce the MFN value.

Future Research Emphasis

As with most research projects, questions are raised as the research progresses, and certain procedures and concepts can be altered to improve the results. Several possible areas that could be studied in the future will be presented.

A first suggestion for future research would be a similar study as the one reported in this dissertation except using a different experimental unit in order to reduce intake and cecal enlargement problems. The chicken has been noted for a relative constancy of intake even with diets low in protein. However, the anatomical arrangement of the ceca and large intestine could reduce the chicken's usefulness. The pig also tends to keep intake at near normal levels with lower protein diets. The swine nutrition research group at Iowa State University

has recently developed a very successful cecal cannulation procedure which would allow for easy and continuous sampling of cecal contents. However, the large quantities of egg protein or other high quality proteins needed could be prohibitive for feeding a large number of pigs. Based on the results in this dissertation, it may be better for future similar projects to only concentrate on the lower protein diets.

In any of the animals mentioned, some effort should be directed towards the utilization of nonprotein nitrogen for the satisfaction of the microbial requirement for nitrogen. Also, supplemental methionine should be investigated to determine if nitrogen losses would be modified. As discussed previously, any modification of nitrogen losses by these two factors would only be expected at low protein intakes. Antibiotics could be used to modify intestinal microbial populations, but the use of germfree animals might provide increased responses. However, the facilities needed for germfree work could be a drawback to their use.

To determine if these effects are similar in ruminants, lambs or calves could be maintained on liquid diets to reduce the functionality of the rumen. As pointed out previously any ruminal fermentation and resultant microbial synthesis could mask fermentation effects in the lower intestine. Without the ruminal fermentation influence large intestine and cecal fermentation could be investigated.

Another possible experimental animal would be the horse. The large intestine of the horse appears to have a greater capability to absorb amino acids than the large intestine in other animals. Therefore,

effects as proposed in this dissertation may be magnified in the horse. Also, those results may help to more critically design research with other animals where the large intestine is not as functional. Recent research has shown that the adult horse can effectively utilize urea for its maintenance nitrogen requirement, but similar research needs to be conducted with the young growing horse.

Also, basic *in vitro* and *in vivo* projects need to be carried out to investigate the absorption of amino acids in the lower intestine including the ileum, cecum, and colon. This research should be done at low protein levels with and without normal microbial populations.

Lastly, the effect of the lower intestinal microflora should be investigated in animals such as cows, ewes, and some sows normally fed poorer quality roughages. Normally these animals are limited in nitrogen intake, but large amounts of fermentable energy are presented to the lower intestine. If the fermentation in the large intestine is increased, it could mean an additional loss of nitrogen in the feces from intestinal endogenous nitrogen. Use of urea to satisfy the lower intestinal microbes' nitrogen requirement seems to be a possible first approach. Also, as research into monensin supplementation in cows increases there is the possibility it could modify the lower intestinal microflora. Also, if methionine supplementation is of benefit in reducing the nitrogen loss at low protein levels in laboratory animals, protected proteins containing high levels of sulfur amino acids should be investigated in mature ruminants. If nitrogen losses could be

substantially reduced, or covered with a cheaper source of dietary nitrogen, economical benefits to the producer raising cows, ewes, or sows could be realized. Little economic benefit would be expected in most feedlot situations due to the urea already recycling into the digestive tract with the higher protein diets.

SUMMARY

The objective of the research presented in this dissertation was to investigate the origin of the metabolic fecal nitrogen (MFN) in light of new concepts of microbial fermentation in the lower intestine. Detailed systems of protein nutrition in ruminants have led to a greater understanding of the relationship of the ruminal fermentation to the host animal's protein nutrition. Although some of these systems require MFN values, little direct research into the influence of lower intestinal fermentation on that source of nitrogen loss has been reported. A few reports in the literature indicate the intestinal microbial microflora increase the MFN loss. Additional reports indicate that germfree and antibiotic supplemented animals grow faster and excrete less total fecal nitrogen with the majority as nonprotein nitrogen. The present study utilized a high quality protein in diets fed to laboratory rats having either a normal intestinal microflora or one modified by antibiotics. At higher intakes of nitrogen the modification of the microflora had little effect as compared to the normal microflora. When no dietary protein was fed rats with the modified intestinal microflora had increased carcass gain and reduced total carcass nitrogen loss. The loss in total carcass nitrogen was approximately 50% of that predicted by current requirement standards. Also, the total carcass nitrogen was quite similar to that predicted by a regression equation calculated from data at higher levels of protein. The nitrogen

composition of cecal contents and the movement of the same nitrogenous compounds across the intestine indicate the possibility of greater amino acid absorption in the modified intestinal microflora rats fed no protein as compared to the normal counterparts. Therefore, based on these data from the present study and from an extensive literature search, it can be concluded that the microflora of the lower intestine do have an effect on fecal nitrogen losses and most especially on the MFN loss. The influence of the microflora on the MFN loss is extensive enough to modify the currently accepted Thomas-Mitchell biological value determination. At higher protein intakes the true MFN, the body α amino nitrogen lost in the feces, is suggested to be lower than presently accepted, especially in ruminants. The use of MFN to determine a biological value for a protein to be fed at high levels of intake overestimates the usefulness of the protein at that intake. Usage of accepted quantities of MFN in newer protein feeding standards should be critically evaluated, and the MFN value reduced or not used at all.

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APPENDIX

Table A1. Effect of modified intestinal microflora on average daily gain and final weight at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Average daily gain			
			Mean[c] g	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<
0.00	-	7	-1.60	±0.16	.20	-
	+	7	-1.30	±0.18		-
2.62	-	11	-0.25	±0.12	.003	.001
	+	11	+0.26	±0.12		.001
5.25	-	9	+1.24	±0.15	.001	.001
	+	11	+2.00	±0.12		.001
7.88	-	3	+2.10			
	+	3	+3.14			
10.50	-	9	+3.95	±0.13	.001	.001
	+	10	+5.16	±0.12		.001
15.75	-	8	+4.69	±0.14	.001	.001
	+	4	+5.58	±0.19		.08
21.00	-	3	+4.57			
	+	5	+5.88			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Final weight			
		Significance level[b]	
Mean[c] g	SE	Antibiotic[d] P<	Protein[e] P<
101.87	±2.84	.06	-
109.64	±3.37		-
121.44	±2.11	.02	.001
128.67	±2.10		.001
142.82	±2.88	.005	.001
152.86	±2.12		.001
158.67			
173.67			
177.56	±2.43	.001	.001
197.54	±2.22		.001
191.52	±2.60	.01	.001
202.94	±3.46		.20
190.27			
203.88			

Table A2. Effect of modified intestinal microflora on carcass gain, carcass weight, and carcass nitrogen at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Carcass gain Mean[c] g	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<
0.00	-	7	-32.31	±2.83	.05	-
	+	7	-23.91	±3.35		-
2.62	-	11	-5.31	±2.10	1.00	.001
	+	11	-4.59	±2.09		.001
5.25	-	9	+18.55	±2.86	.45	.001
	+	11	+16.11	±2.11		.001
7.88	-	3	+27.78			
	+	3	+27.37			
10.50	-	9	+51.17	±2.42	.45	.001
	+	10	+54.22	±2.21		.001
15.75	-	8	+60.57	±2.59	.25	.02
	+	4	+55.77	±3.45		.85
21.00	-	3	+53.42			
	+	5	+58.15			

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Carcass nitrogen (wet basis) Mean[c] %	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<
0.00	-	7	3.38	±0.04	.75	-
	+	7	3.40	±0.05		-
2.62	-	11	3.39	±0.03	.85	.95
	+	11	3.38	±0.03		.70
5.25	-	9	3.31	±0.04	.90	.15
	+	11	3.31	±0.03		.15
7.88	-	3	3.45			
	+	3	3.45			
10.50	-	9	3.33	±0.04	.55	.75
	+	10	3.36	±0.03		.40
15.75	-	8	3.40	±0.04	.45	.15
	+	4	3.45	±0.05		.15
21.00	-	3	3.50			
	+	5	3.36			

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Carcass weight			
		Significance level[b]	
Mean[c] g	SE	Antibiotic[d] P<	Protein[e] P<
79.18	±2.89	.05	-
87.60	±3.42		-
105.80	±2.14	1.00	.001
105.91	±2.14		.001
129.61	±2.92	.45	.001
126.85	±2.15		.001
143.40			
142.99			
161.78	±2.47	.45	.001
164.58	±2.25		.001
171.12	±2.64	.25	.02
165.61	±3.52		.85
165.17			
166.16			

Total carcass nitrogen			
		Significance level[b]	
Mean[c] g	SE	Antibiotic[d] P<	Protein[e] P<
2.68	±0.09	.02	-
2.98	±0.11		-
3.58	±0.07	.95	.001
3.57	±0.07		.001
4.28	±0.09	.45	.001
4.19	±0.07		.001
4.95			
4.94			
5.38	±0.08	.20	.001
5.52	±0.07		.001
5.83	±0.08	.45	.001
5.71	±0.11		.15
5.77			
5.58			

Table A3. Effect of modified intestinal microflora on nitrogen retention, Thomas-Mitchell and new biological values at different dietary protein levels

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Nitrogen retention Mean[c] g	SE	Significance level[b] Antibiotic[d] P<	Significance level[b] Protein[e] P<	Mean[c] %
0.00	-	7	-0.83	±0.09	.03	-	-[f]
	+	7	-0.53	±0.11		-	-
2.62	-	11	0.08	±0.07	.90	.001	129.75
	+	11	0.10	±0.07		.001	92.77
5.25	-	9	0.78	±0.09	.55	.001	109.33
	+	11	0.71	±0.07		.001	85.73
7.88	-	3	1.41				101.80
	+	3	1.40				86.83
10.50	-	9	1.90	±0.08	.20	.001	91.24
	+	10	2.05	±0.07		.001	83.91
15.75	-	8	2.35	±0.09	.50	.001	70.99
	+	4	2.25	±0.11		.15	64.28
21.00	-	3	2.19				56.23
	+	5	2.21				49.57

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared only to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

[f]No values computed for this protein level.

Thomas-Mitchell biological value			New biological value			
	Significance level[b]				Significance level[b]	
SE	Antibiotic[d] P<	Protein[e] P<	Mean[c] %	SE	Antibiotic[d] P<	Protein[e] P<
			-[f]			
			-			
±4.89	.001	-	99.24	±4.68	.40	-
±4.87		-	93.37	±4.66		-
±6.81	.005	.02	93.85	±6.51	.35	.50
±4.91		.35	85.76	±4.69		.30
			94.28			
			86.83			
±5.65	.35	.04	83.81	±5.40	1.00	.25
±5.14		.80	83.68	±4.91		.80
±6.04	.55	.02	66.61	±5.77	.85	.04
±8.02		.05	64.21	±7.68		.04
			52.01			
			49.57			

Table A4. Effect of modified intestinal microflora on total cecal ammonia, urea, and free amino acids at different levels of dietary protein

Dietary crude protein %	Antibiotic supplementation[a]	Number of rats	Cecal ammonia Mean[c] µmoles	SE	Significance level[b] Antibiotic[d] P<	Protein[e] P<	Mean[c] µmoles
0.00	-	5	27.67	±22.02	.65	-	8.67
	+	5	43.77	±26.98		-	26.62
2.62	-	9	19.86	±14.64	.001	.80	4.78
	+	8	130.66	±15.73		.008	50.96
5.25	-	9	21.59	±14.64	.001	.95	5.26
	+	9	224.14	±14.64		.001	78.37
7.88	-	3	16.08				4.24
	+	3	456.49				83.00
10.50	-	8	35.99	±15.76	.001	.55	6.22
	+	8	442.52	±15.76		.001	87.37
15.75	-	6	67.32	±19.33	.001	.25	2.58
	+	3	511.79	±25.76		.03	106.51
21.00	-	2	85.35				12.33
	+	2	333.03				72.07

[a]None supplemented = -, supplemented = +.

[b]Interpretation example Table 7.

[c]Least square mean ± least square standard error. Actual mean only and no statistics reported for protein level 7.88 and 21.00 since levels not consistent across trials.

[d]Antibiotic effect within each protein level.

[e]Protein effect within antibiotic supplementation (+ or -), but compared to the adjacent lower protein level, excluding 7.88 and 21.00 protein levels.

Cecal urea			Cecal free amino acids			
	Significance level[b]				Significance level[b]	
SE	Antibiotic[d] P<	Protein[e] P<	Mean[c] µmoles	SE	Antibiotic[d] P<	Protein[e] P<
± 8.85	.20	-	69.88	± 82.31	.30	-
±10.84		-	192.86	±100.85		-
± 5.88	.001	.75	43.32	± 54.72	.001	.80
± 6.32		.06	586.83	± 58.79		.002
± 5.88	.001	1.00	47.93	± 54.72	.001	1.00
± 5.88		.003	966.16	± 54.72		.001
			33.21			
			1434.96			
± 6.33	.001	.95	36.57	± 58.91	.001	.90
± 6.33		.35	1326.72	± 58.88		.001
± 7.76	.001	.75	68.46	± 72.24	.001	.75
±10.35		.15	1473.08	± 96.31		.25
			96.76			
			842.82			